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THE CLINICAL AND PATHOLOGIC EFFECTS OF THE NITROGEN AND SULFUR MUSTARDS IN LABORATORY ANIMALS*

IRVING GRAEF, M.D., DAVID A. KARNOFSKY, M.D., VAL B. JAGER, M.D.,
BORIS KRICHESKY, Ph.D., AND HOMER W. SMITH, Sc.D.

*(From the Departments of Physiology and Pathology, New York University
College of Medicine, New York 16, N.Y.)*

The sulfur (bis- β -chloroethyl sulfide) and nitrogen mustards (alkyl secondary and tertiary β -chloroethyl amines) have been investigated for their pharmacologic action, toxicity, mechanism of action, clinico-pathologic effects, and the therapy of their effects. Other reports¹⁻⁴ have dealt or will deal with their pharmacology, toxicity, and certain physiologic and therapeutic aspects. This report is concerned chiefly with their clinical and pathologic effects. Due to restrictions imposed by wartime security requirements, many of the data available in classified documents of other American, Canadian, and British investigators can be cited only on the basis of the original reports which are not yet available in the open literature. Where priority of observations is concerned, these reports are cited as dated personal communications.

Attention was focused in our work on the clinical and pathologic effects of the following compounds (designated by the official War Department symbols for brevity); the essential toxicity data are taken from Anslow, Karnofsky, Jager, and Smith.^{1,2}

H = bis(β -chloroethyl) sulfide

HN₁ = ethyl-bis(β -chloroethyl) amine

HN₂ = methyl-bis(β -chloroethyl) amine

HN₃ = tris(β -chloroethyl) amine

TL 301 = iso propyl-bis(β -chloroethyl) amine

Since our most complete studies were made on HN₂, methyl-bis (β -chloroethyl) amine, the results are reported in detail for this compound as the prototype for these vesicants. Where deviations occurred in identical tests with the other agents, the illustrative data are included.

There is ample evidence that the nitrogen compounds resemble H in

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their biologic effects. The free bases are vesicant: capable of injuring all tissues with which they come in contact in sufficient dosage. Their systemic effects are notably similar in time relations and in the selected tissues that are most distinctively affected.

SECTION I CLINICAL EFFECTS

The original observations reported herein have been confined principally to rodents (albino mice, albino rats, and New Zealand rabbits obtained from Carworth Farms) and some dogs. Where significant data are available, reference is made to the effects of the compound in other species.

Influence of Route of Administration

The *subcutaneous*, *intravenous*, *intramuscular*, and *intraperitoneal* routes of administration provide accurate modes of giving measured dosages of candidate compounds for toxicologic appraisal. When volatile substances are tested *percutaneously*, the loss of agent, by evaporation or local fixation, introduces considerable variation when correlating dosage with systemic effects. Obviously, changes in environmental temperature and humidity affect vaporization through their influence on skin temperature. Rates of absorption are also influenced by skin temperature, moisture, and the rate of penetration of each compound.⁵

The appraisal of mortality and clinicopathologic effects *via inhalation* of the vapor, as in the case of HN₁, HN₂, and H, is especially difficult because of the unknown quantity of inhaled dose. In the case of a nonvolatile agent dispersed as a particulate (as has been necessary with HN₃ until very recently) the measure of concentration in a gas-sing chamber as a point of reference for the inhaled dose is especially dubious. It is probably better than none, but one should not overlook the factors that may promote or retard inhalation and absorption of a particulate, *viz.*, particle size, the degree of impaction and arrest in the nose, variations in respiratory rate and volume, absorption from the nose, species variations, and, particularly, impaction on the body fur necessitating efforts or devices to prevent licking and oral ingestion of agent from contaminated fur.

It is important to note that the LD₅₀ is an arbitrary statistical value, encompassing a wide, often unaccounted for, range of variation and even different modes of death. At the LD₅₀, 5 of 10 intoxicated animals may be expected to die; at 0.5 LD₅₀ only one of 10 may be lethally intoxicated; yet the 5 animals dying at the LD₅₀ and the single animal dying at 0.5 LD₅₀ may show the same degree of pathologic injury.

Similarly, within the 10 animals receiving an LD₅₀ dose, the pathologic changes may range from almost nil to the most extreme. Hence the actual dose administered may sometimes be of little value in estimating biologic effect; each animal must be appraised individually on the basis of its particular symptoms and pathologic changes.

The form of the compound had to be varied for certain routes of administration. For gassing and cutaneous experiments the free bases were used; for intravenous and subcutaneous injections the HCl salts of the nitrogen mustards were employed. We have assumed, without direct proof, that the injection of the HCl salts was equivalent to the injection of the free base.

The problems presented by the intravenous dosage of H have been especially vexing. The literature from World War I to date on the toxicity of H is inconsistent. This is not surprising when one realizes the difficulties of administering this compound intravenously. After gassing or skin application the results are fairly consistent, if licking or swallowing of H is avoided. The subcutaneous route also yields a uniform LD₅₀ and pattern of systemic effects. But the intravenous route has been unpredictable. We have observed considerable variation in the LD₅₀ of H via this route which we believe to be due to the mode of administration. When H is given neat and slowly, the LD₅₀ for rabbits is about 4.8 mg. per kg., but when given rapidly it is 3.6 mg. per kg. When given in propylene glycol so that the volume of the solvent is 0.5 cc. per kg. of rabbit the LD₅₀ falls to 2.7 mg. per kg. When given neat, H lodges in the pulmonary capillaries, and an indefinite amount reacts locally and is not available to produce systemic injury. Neat H is immiscible with the blood and when given intravenously the droplets act like fat emboli and are distributed according to size, rate, and number of droplets introduced. Pulmonary injury going on to infarction and necrosis was found even after the administration of supra LD₅₀ doses of H dissolved in propylene glycol. Earlier workers⁶⁻¹⁰ also have reported pulmonary injury after H but did not relate it to the toxicity of the compound or account for its pathogenesis. To avoid pulmonary injury by H droplet-embolization we have administered the agent dissolved in propylene glycol, and at dosages in which toxicity was least likely to be affected by pulmonary deposits.

To avoid comparisons with animals dying from acute neurotoxic injury (within 8 hours or less), or from late complications (after 5 days), most of the animals were killed at the point of death or sacrificed at 72 or 96 hours in the comparative study of pathologic effects. A few animals examined at 48 to 120 hours are included in some of the tabulations.

Individual Pharmacologic Actions

For general interest, a summary of the individual pharmacologic actions is given but the reader may consult Gilman and Phillips¹¹ for further comments. Above LD₅₀ doses, certain characteristic pharmacologic actions are manifest chiefly on the nervous system. They are readily elicited on intravenous administration, less readily on administration by other routes.

Cholinergic action is possessed by both H and HN₂, and is characterized by "muscarinic" action on glands and smooth muscle and by "nicotinic" action on autonomic ganglia and skeletal muscle. In the case of both agents, peripheral stimulation appears to account for the "muscarinic" responses, although central excitation, apparently minimal, cannot be excluded. Cholinergic action is not considered a factor in the development of the syndrome leading to delayed death, and *is not responsible for late vomiting and diarrhea*. Whether or not cholinesterase inactivation is involved in this cholinergic action has not been determined.

Parasympatholytic action is shown by HN₃ at small doses, as judged by paralysis of cardiac vagal fibers and antagonism of the action of parasympathomimetic drugs. A similar parasympatholytic action of H, HN₂, and TL 301 appears to be present in severe intoxication.

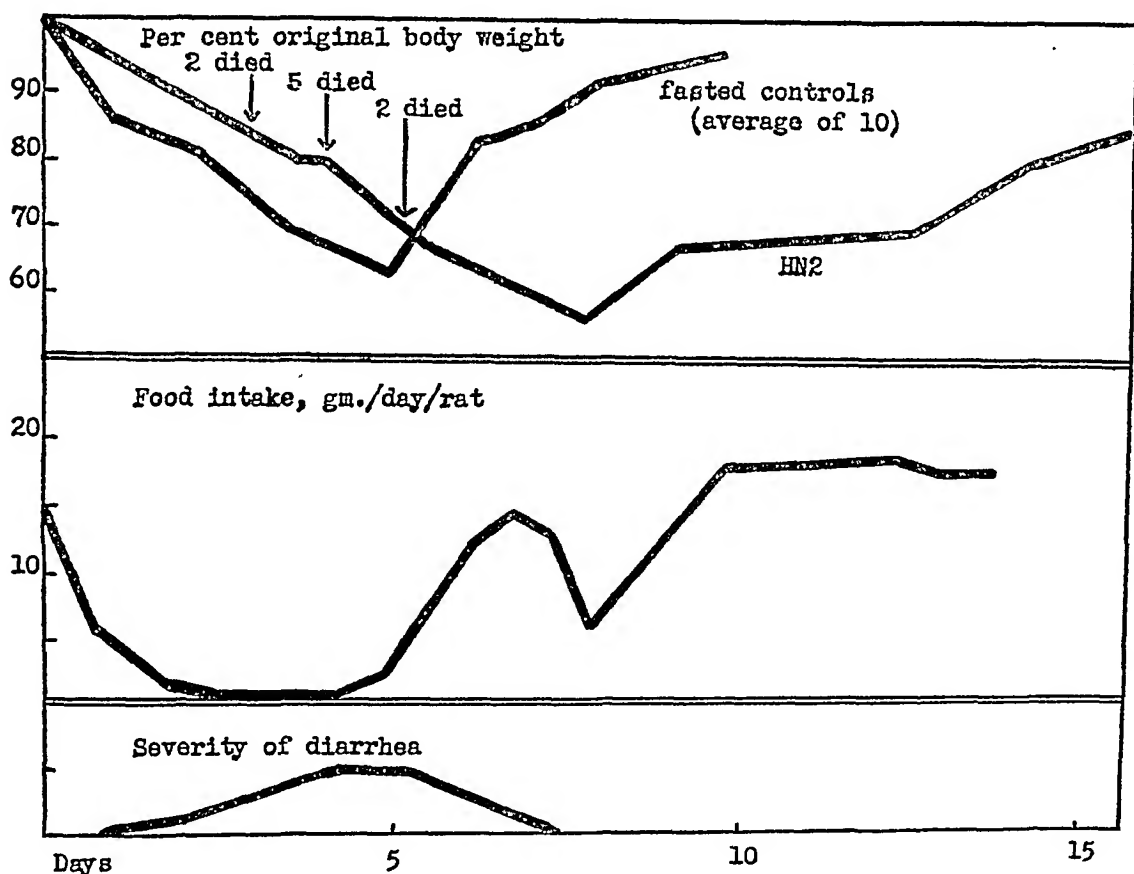
Convulsive action is so distinctive of HN₃ as to warrant classification of this agent as a true convulsive drug. High doses of this compound given parenterally have an immediate, direct, intense convulsive action which results in death within 6 hours. Central stimulation is a property of H and HN₂ at high doses, but the action is not similar to that of HN₃.

Paralytic action is possessed by both HN₂ and HN₃. This action is characterized by a progressive, irreversible muscular weakness and may cause death in a number of hours.

Neurologic injury has been observed in animals intoxicated with HN₁ and HN₂ by either gassing or intravenous administration, but never after intoxication by other routes or with the other agents. This injury has usually appeared about the third or fourth day in animals showing no other evidence of systemic intoxication. In extreme cases, the injury is fatal, but among survivors hyperirritability remains for weeks.

Neurologic Death. Large doses given parenterally (5 to 10 LD₅₀ or more) induced neurologic injury and death in a few hours, the outstanding manifestations of intoxication consisting of convulsions, depression, loss of coordination, irritability, tremors, weakness, dyspnea,

evidence of parasympathomimetic activity—salivation, lacrimation with red tears in rats, defecation, and urination—and terminal respiratory paralysis. At the lower doses producing neurologic effects, animals survive up to 40 hours and also show evidence of delayed systemic intoxication. (Intravenous injection induces neurologic signs at lower doses, relative to the LD_{50} , than subcutaneous injection.)



Text-Figure 1. Data on 10 rats receiving 1.7 mg. per kg. of HN_2 intravenously ($1.5 LD_{50}$).

Delayed Death from Systemic Intoxication. Mice, rats, rabbits, and dogs, receiving 1 to 3 LD_{50} parenterally, may remain relatively asymptomatic for 24 to 48 hours, except for mild anorexia and slight weight loss. Following this latent period debilitation is rapid (Text-Fig. 1). The fur becomes ruffled and the animal huddles in a corner; it stops eating and drinking and becomes progressively weaker, although somewhat hyperirritable, and develops watery diarrhea. The body temperature falls and there is rapid decrease in body weight, partly attributed to the loss of fluid through the intestinal tract. The animal becomes progressively more depressed, apathetic, and unresponsive as death approaches. In rabbits the symptoms of intoxication are not as marked; and although eating and drinking stop and mild diarrhea develops, weight loss is not as severe and the animal remains

fairly responsive until shortly before death. Severe leukopenia is present in all four species at death. A dog receiving 2.5 mg. per kg. of HN₂ intravenously was relatively asymptomatic until just prior to death on the fourth day. About 5 mg. per kg. of HN₂ in dogs was fatal in 2 to 4 days, inducing vomiting, incoordination, bloody diarrhea, repeated convulsions, and anorexia prior to death.

At autopsy, intoxicated animals appear emaciated and dehydrated. The stomach is distended with food and fluid, and the small intestine, especially the duodenum, is distended with yellowish fluid which is occasionally mucoid in nature, and the intestinal wall is transparent and friable. Small hemorrhagic and hyperemic areas may occur in the wall of the small intestine, and ulcerations of the mucosal surface of the distal part of the ileum are noted occasionally. In rats, Peyer's patches appear hyperemic and the cecal lymph nodes are frequently hemorrhagic. The cecum is usually filled with food and fluid, and the colon is often empty or contains mucus, gas, or fluid feces (notably in rabbits). In rats the adrenal glands are markedly enlarged. In dogs, the intestinal lesions are complicated by hemorrhagic ulcers.

The spleen is small and comparatively bloodless, the thymus is atrophic, and the lymph nodes sometimes appear hemorrhagic or are so greatly reduced that they are difficult to identify. The bone marrow is either red and liquid, or fatty in appearance.

Fowler and Grant¹² have described pulmonary edema in guinea-pigs after subcutaneous, but not after intraperitoneal, injection of HN₂ hydrochloride.

This clinicopathologic picture is designated in this report as *delayed death* from systemic intoxication, and is to be distinguished from deaths occurring 7 days or longer after intoxication, the specificity of which is in question, and which we designate as *remote deaths*.

Remote Deaths. While most mice and rats receiving LD₅₀ doses parenterally and surviving 7 days effect a complete recovery, in a few weight loss continues and death may occur as long as 20 days after injection. In such animals, weight loss is severe, in the range of 30 to 50 per cent of the original body weight. Hodge *et al.*¹³ have shown in acute starvation that 70 per cent of 3-months-old mice die within 4 days, after an average weight loss of 30 per cent, although older mice under the same conditions may live slightly longer; while Mulinos and Pomerantz¹⁴ have found that weight losses of 35 to 45 per cent may be fatal in rats. Most of our animals with remote deaths were in this dangerous range of weight loss and therefore death cannot be charged specifically to the late effects of HN₂. In many instances infection was an obvious complication, and it may be suspected that, in others, less

evident infection contributed to the persistence of anorexia and progressive debilitation. The possibility of a direct lethal action effective beyond 7 days appears unlikely.

After the crisis of intoxication which occurs between 70 and 120 hours, there ensues a rapid recovery of the leukocyte count and a slow regain of weight. Wilson, Vars, and Gurin¹⁵ have noted that rats surviving a dose slightly greater than the LD₅₀ occasionally regain weight but remain extremely apprehensive and hyperirritable. The same phenomenon was sometimes noted in our rats. Mice surviving LD₅₀ doses are capable of bearing normal young within a few weeks after intoxication. Observations on this point have not been made in other species.

Effects of Different Routes of Administration

By cutaneous application of the *free base*, the LD₅₀ of HN₂ is reported as 35 mg. per kg. for mice,¹⁶ 14 to 15 mg. per kg. for rats,^{17,18} 12 mg. per kg. for rabbits,¹⁷ 20 mg. per kg. for goats,¹⁷ and about 50 mg. per kg. for monkeys.¹⁸ The clinical picture, when recorded, is typical of systemic intoxication. The factors of skin thickness, temperature, local fixation, evaporation, and the rate of penetration of the applied liquid vary with the species, the site of application, and the environment, so that toxicity figures are chiefly of value for comparison with the cutaneous LD₅₀ of other compounds.

The LD₅₀ of HN₂ hydrochloride by subcutaneous (2.6 mg. per kg.²) or intraperitoneal (about 2.3 mg. per kg.²) injection in mice is not more than 50 per cent greater than the *intravenous* LD₅₀ (about 2.0 mg. per kg.²). The free base appears to be somewhat less toxic.¹⁹ There are no marked differences in susceptibility among mammals.²

The influence of ingested food on the oral LD₅₀ for HN₂ was shown as follows. Fed mice receiving orally an LD₅₀ solution of the hydrochloride (0.4 cc. of a 45 to 250 mg. per cent solution in saline) die over a period of 4 to 7 days and the symptomatic picture and changes at autopsy are typical of delayed death. The LD₅₀ was 20 mg. per kg. Mice receiving higher doses (50 mg. per kg.) developed severe hemorrhagic and ulcerative lesions in the duodenum and jejunum, with occasional perforations, and deaths occurred within 2 to 3 days. In fasted mice (*i. e.*, deprived of food for 18 to 24 hours before the intrasophageal administration of the hydrochloride, and then re-fed shortly afterwards) the LD₅₀ is 10 mg. per kg. The great majority of deaths occurred within 2 to 3 days, the animals showing at autopsy extremely severe changes in the duodenum and jejunum with hemorrhage, ulceration, and in some cases perforation. Mice surviving this period usually

recovered. It is concluded that food and gastrointestinal secretions detoxify or bind part of the HN_2 and thus limit local damage; this process, however, does not appear to prevent the absorption of the compound in a toxic form.

Mice and rats gassed at 1 to 2 LC_{50} (0.3 to 0.6 mg. per liter for 10 minutes) develop dyspnea, wheezing, nasal secretion and opaqueness of the cornea, and death usually occurs 72 to 144 hours after gassing, with the picture of systemic intoxication. Rabbits have more severe respiratory symptoms, but systemic absorption of the compound, nevertheless, contributes largely to death. Remote deaths are common in animals surviving this critical period, but these are scattered in a random manner over the period of 5 to 20 days; such animals continue to lose weight and frequently show persistence of respiratory difficulties. Respiratory infection unquestionably plays an important part in these remote deaths and rats that have recovered their initial body weight may continue to show excessive nasal secretion for 3 to 4 weeks after gassing.

Mice gassed at 2 to 3 LC_{50} develop severe neurologic disturbances with agitation, ataxia, and incoordination, these symptoms diminishing within 48 hours. Most of the deaths do not occur until 72 to 100 hours after gassing. Rats also show neurologic symptoms at this dose, but die within 40 hours, apparently of a combination of neurologic, respiratory, and systemic injury. Neurologic manifestations at low multiples of the LC_{50} , an occurrence not noted after equivalent subcutaneous doses, have also been described by the University of Chicago Toxicity Laboratory.²⁰

Dogs, cats, and goats are reported to suffer from such severe respiratory injury in the way of edema of the glottis, necrosis and sloughing of the epithelium of the trachea and bronchi, with pulmonary injury (which may in some cases be secondary to infection), that they undergo an early respiratory death^{20,21} rather than the delayed death (about 100 hours) associated with systemic intoxication and typical of gassed mice and rats. This may explain why the LC_{50} in larger animals is significantly lower than in mice, rats, and rabbits.

The vapor causes redness of exposed surfaces and occasionally edema of the ears and male genitalia of mice, rats, and rabbits, but severe cutaneous lesions are not produced at LC_{50} doses.

SECTION II

SYSTEMIC EFFECTS OF HN_2 AND H

Irrespective of the route by which HN_2 or H enters the body of mice, rats, and rabbits (percutaneously, subcutaneously, intravenously,

orally, or by gassing), they exert certain qualitatively consistent pathologic effects in doses at or moderately above the LD₅₀, notably affecting the blood-forming organs and small intestine. In many cases these systemic effects appear to be more important in causing death than the local injury and they will be described without specific reference to the route of administration. The susceptibility of the tissues affected was appreciated during and after World War I.^{6-8,22,23} In this report we have omitted detailed consideration of the local effects of these compounds for the sake of brevity.

Routine pathologic examination has consisted of the study of the larynx, trachea, and lungs, several levels of the esophagus, stomach and intestinal tract (longitudinally rolled segments of the entire intestinal tract were prepared in mice, rats, and some rabbits; in dogs large pieces were similarly rolled to provide adequate samples), liver and gallbladder, pancreas, kidneys and urinary bladder, spleen, thymus, lymph nodes, femoral and sternal bone marrow, testes and ovaries, thyroid, adrenals, and heart muscle. Occasional specimens of the eyes and nose were studied in cross sections of the entire head.

Tissues were obtained by sacrifice or at death, and routinely fixed in Zenker's fluid, cut at 6 μ , and stained with hematoxylin and eosin. Sections of the spleen and bone marrow were stained with Giemsa's stain, or eosin azure after Maximow. Sections of the marrow were cut at 3 to 4 μ . Special stains for iron and fats were used occasionally.

HEMATOLOGIC EFFECTS OF HN₂

Leukopenia, the most distinctive feature of intoxication yet described, has been found in all species studied: mouse, rat, rabbit, guinea-pig, dog, cat, goat, chicken, pigeon, monkey, and man. It was first reported in gassed mice.²⁰ We have followed the course of hematopoietic injury in mice, rats, and rabbits by means of daily total and differential counts. The leukocyte counts were done in the usual manner, the smears being stained with Wright's or the Jenner-Giemsa stains.

Mice

One hundred leukocyte counts and 75 differential counts were made on specimens of tail blood of mice before and after subcutaneous administration of the HN₂. Our normal average leukocyte count (based on the examination of 52 tail bloods) was 26,150 (range, 12,250 to 49,000) per cmm. Leukocyte counts on heart blood have been reported to range from 4,000 to 6,000.²⁴ Differential examination was somewhat more consistent, but variations were wide. About 10 per cent of the erythrocytes showed polychromasia, an incidence recorded by others.²⁵

Because of great variations in the normal tail blood, the mouse is not regarded as a satisfactory subject for routine or quantitative hematologic studies. Therefore only the unequivocal changes found in intoxicated animals are presented.

Subcutaneous administration of 30 to 40 mg. per kg. of HN_2HCl was fatal in 12 to 40 hours. Leukocytosis was present within 12 hours, but in the animals surviving longer the count had begun to fall. The leukocytosis was due entirely to an increase in granulocytes, since lymphopenia occurred early. Polychromasia was not affected. The platelets on the smear appeared to be increased in numbers, although no counts were done.

Administration of 3.0 to 8.0 mg. per kg. subcutaneously was fatal in 72 to 144 hours. Lymphopenia with granulocytosis of variable degree appeared within 12 hours and was followed by a precipitous decline in the total leukocyte count. Four days after injection the average leukocyte count was 3,000 and polychromasia was reduced or absent.

TABLE I

Effects of 3.0 mg. per kg. of HN_2HCl Given Intravenously in Albino Rats

Hours after injection	No. observed	White blood cell count	Differential count				
			Granulocytes		Small lymphs	Large lymphs	Mono-cytes
			Banded	Segmented			
Control	34	per cmm. 10,000 (7,000-15,000)	per cent 2 (0-5)	per cent 23 (10-31)	per cent 68 (52-80)	per cent 5 (2-12)	per cent 2 (0-6)
24 hours	8	3,400 (1,150-7,100)	0	74 (68-84)	25 (14-33)	0	1 (1-2)
48 hours	14	2,300 (50-5,800)	1 (0-9)	88 (70-97)	9 (0-23)	2 (0-8)	0
72 hours	10	600 (250-1,150)	Too few cells for differential counts, but 90 per cent of the cells seen were lymphocytes				

Subcutaneous doses of 1.5 to 2.5 mg. per kg., permitting many recoveries, produced a temporary fall in the leukocyte count which returned to normal in 4 to 6 days. Polychromasia was decreased on the fourth to sixth day, followed by a definite increase above normal.

Mice gassed at 1 to 3 LC_{50} and dying 3 to 6 days later also showed a severe leukopenia as reported by Lushbaugh at the University of Chicago.^{20,26}

Rats

Blood for leukocyte counts and differentials was obtained by cardiac puncture from unanesthetized rats and at sacrifice from the free-flowing aortic blood of anesthetized animals. Control counts and differentials

(Table I) were in agreement with the data of others.^{27,28} Rats were given 3 mg. per kg. of the hydrochloride intravenously (2.5 LD₅₀) in one series and 3.0 mg. per kg. subcutaneously (1.6 LD₅₀) in another series. Animals in both groups died in the period of 72 to 110 hours after administration. The rapid fall in leukocytes and the change in the differential count were almost identical in the two groups and the data obtained on the intravenous group are presented in Table I.

Rats near death at 3 to 6 days after gassing at 1 to 4 LC₅₀ showed a severe leukopenia, as also noted by Lushbaugh.²⁶

Rabbits

Our most extensive study of the circulating blood was made on rabbits. The material is presented in some detail although it represents an incomplete study. It is organized, however, for the purpose of orientation in the problem and toward the planning of further hematologic studies.

Normal Leukocyte and Differential Counts in the Rabbit. Blood from the ear vein or occasionally blood taken by cardiac puncture was used. Conditions tending to produce fluctuations in the leukocyte and differential counts could not be eliminated from our study. It is known that there is a moderate variation in leukocyte count during the day; that heart blood and venous blood obtained from the ear differ in leukocyte and differential count unless the venous blood is flowing freely; and that rabbits placed on their back (*i.e.*, for cardiac puncture) show a progressive fall in leukocyte count because of a decrease in lymphocytes.²⁹ However, our control counts, established on 21 rabbits under a variety of conditions, were in agreement with the normal counts found by previous investigators³⁰⁻³² and the changes produced during intoxication were far out of the range of variations attributable to our unstandardized methods of obtaining blood.

Twenty-one control leukocyte counts averaged 8,400 (3,650 to 17,500) per cmm. Differential counts were made on the basis of the following classifications:

Granulocytes

- a. Pseudo-eosinophils (corresponding to neutrophils in man)
 1. Banded cells (containing a single band-shaped nucleus and cytoplasmic granules staining with eosin)
 2. Segmented cells (containing a multilobulated nucleus and granules staining with eosin)
- b. Basophils (containing many coarse granules taking the basic stain)
- c. Eosinophils (easily distinguished by eosinophilic granules many times larger than the granules of pseudo-eosinophils)

Agranulocytes

- a. Lymphocytes
- b. Monocytes. (Although monocytes cannot be distinguished readily from larger lymphocytes except by the use of supravital stains, a large and indented or folded nucleus which may assume many tortuous positions is regarded as distinctive of a monocyte. While reasonably sure of this criterion, since vital stains were not used, the possibility of some error must be admitted.)

Differential counts in the rabbit show considerable variation, evident in our data as well as in those of other investigators.

Supralethal Intravenous Doses. Five mg. per kg. intravenously was fatal to rabbits in 36 to 94 hours and produced consistent effects on the formed elements of the blood. At 24 hours the leukocyte count was little affected, but most of the cells were mature granulocytes, while the lymphocytes were almost absent. At 48 hours leukopenia was severe, with 150 to 4,200 cells per cmm. Animals surviving 72 hours or longer had almost complete leukopenia, the occasional cells being mature granulocytes. An occasional normoblast or immature

TABLE II

Effects of 0.5 mg. per kg. of HN₂ Hydrochloride Given Intravenously in Rabbits

Leukopenic period: 48-72 hours after injection (3 counts)

Total and range of white blood cell count	Differential count				
	Banded	Segmented	Basophils	Lymphocytes	Monocytes
Average and range of per cent of cells	3 (0-6)	30 (10-42)	9 (2-16)	51 (50-52)	7 (6-10)
Total number and per cent of cells	2,300 (1,550-3,000)	50 (0-100)	780 (300-1260)	110 (60-150)	1200 (890-1500)

Recovery period: 96-120 hours after injection (4 counts)

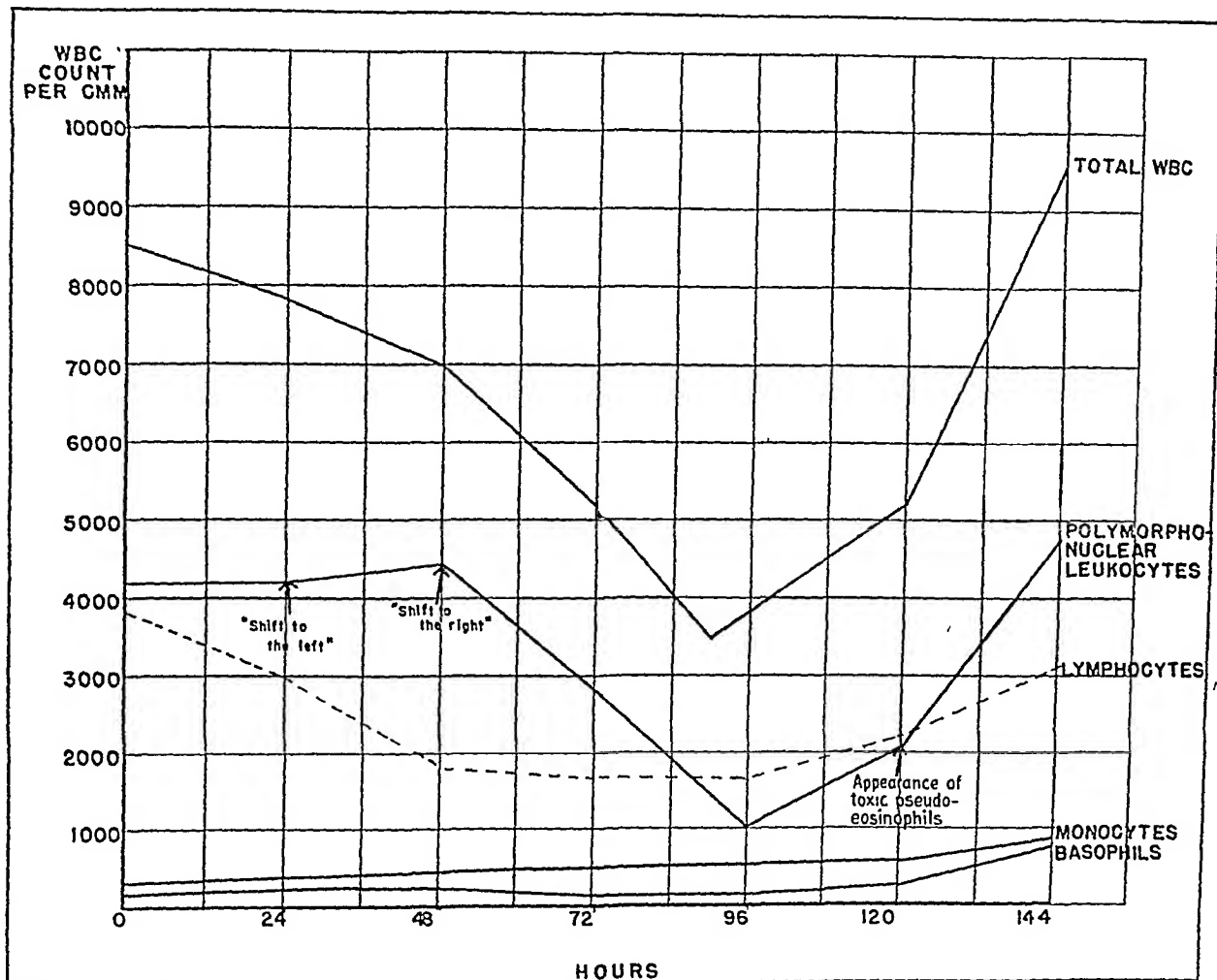
Average and range of per cent of cells	16 (8-32)	30 (15-45)	13 (4-39)	31 (27-59)	10 (8-14)
Total number and per cent of cells	6,350 (4,300-7,800)	1,050 (345-1,840)	2,100 (680-3,500)	800 (230-2,400)	1850 (750-2,650)

granulocyte could be found within 24 hours after injection, but no abnormal forms were seen thereafter. There was a slight progressive fall in hematocrit values.

LD₅₀ Doses. Three mg. per kg. was invariably fatal and 2 mg. per kg. permitted an occasional recovery. Up to 3 to 4 days after injection the course of the leukocyte and differential counts was similar to that seen after 5 mg. per kg., with counts falling to a level ranging from 50 to 660 cells per cmm. In rabbits surviving this period, however, some recovery of the leukocytes was evident. Two rabbits receiving 3 mg. per kg. and surviving longer than 100 hours had "toxic" pseudo-eosinophils,* anisocytosis, macrocytosis, and polychromasia. (One rabbit survived 170 hours and the leukocyte count was 3,000 at the time of death.)

* We refer to polymorphonuclear pseudo-eosinophils which contain large basophilic granulations of irregular size and number, or cells that are swollen and exhibit bizarre nuclei or multisegmented nuclei, or agranular cells with basophilic cytoplasm, as "toxic" pseudo-eosinophils. Pseudo-eosinophils with basophilic granulations were the most frequent and constant form of abnormal cells present in our smears.

At 2 mg. per kg., several rabbits survived beyond 100 hours and in these evidence of recovery of the leukocyte count was more striking. Whereas at 72 to 96 hours the average of 5 counts was 325 cells per cmm., at 120 hours the average of 4 counts had risen to 1,775 (1,170 to 2,400). The differential count showed an increase in basophils,



Text-Figure 2. Effects of 1 mg. per kg. of HN_2 hydrochloride on the leukocyte and differential counts of rabbits.

monocytes, immature granulocytes (which were occasionally vacuolated), and many "toxic" pseudo-eosinophils. Occasional mitotic figures were found in the circulating blood. There were varying numbers of poikilocytes, anisocytes, stippled cells, and normoblasts, and polychromasia was increased.

Sublethal doses. A dose of 0.5 to 1.0 mg. per kg. caused a transient leukopenia followed by a rapid recovery of the leukocyte count. The effects of 1 mg. per kg. were fairly consistent and are shown graphically in Text-Figure 2 and Table II. Although leukopenia was not as severe as after larger doses, there was failure of young regenerating granulocytes to appear in the circulating blood for 72 or more hours

after injection. The lymphocyte count decreased early but not markedly when compared to the later fall in granulocytes. The rapidity of the recovery of the leukocyte count was striking at these doses, in some cases rising from 2,000 to 15,000 overnight. The nature of the cells contributing to recovery are shown in Figure 1, cells of abnormal type occurring in diminishing numbers up to 12 or 14 days after injection. There was no morphologic change in the red cells.

Gilman, Goodman, Phillips, and Allen³³ have described similar hematologic effects after intravenous, subcutaneous, or cutaneous administration of HN₂, and noted that recovery from the leukopenia may be followed by a secondary fall in leukocyte count. They were able to maintain a leukopenic state over a period of 18 days by daily intravenous injections of 0.5 mg. per kg. of the hydrochloride, and a slow but progressive fall in the red cell count also occurred. MacLeod and Rhoads' data³⁴ are also in agreement, and they particularly commented on the great numbers of pseudo-eosinophilic granulocytes containing basophilic granulations which appear during the stage of recovery from leukopenia. Kethley *et al.*³⁵ also induced severe leukopenia by the cutaneous application of the free base at LD₅₀ doses.

Gassing. Our hematologic data on gassed rabbits are limited, but demonstrate several important features. One rabbit exposed to 1.08 mg. per liter for 10 minutes died after 54 hours. At 24 hours the leukocyte count was 16,750; the differential count disclosed 7 per cent banded and 81 per cent segmented pseudo-eosinophils, 2 per cent monocytes, and 10 per cent small lymphocytes. At 48 hours the leukocyte count was 2,300 with only 11 per cent segmented pseudo-eosinophils, 2 per cent basophils, 80 per cent small lymphocytes, and 7 per cent monocytes. These changes in the leukocyte and differential counts parallel those seen after the parenteral administration of lethal doses of the HN₂, and this rabbit apparently died with a considerable degree of systemic intoxication.

Of 3 rabbits gassed at 0.5 mg. per liter for 10 minutes, one died at 5 days, the second at 9 days, and the third recovered. Both dying animals had leukopenia of about 3,000 cells per cmm. with no increase in immature cells at 48 hours. At 96 to 120 hours the leukocytes were increasing rapidly in both animals, and large numbers of immature forms were evident in the smears. One rabbit, at 120 hours, had a count of 18,940 per cmm. with a differential count of 15 per cent myelocytes, 67 per cent banded "toxic" pseudo-eosinophils, 12 per cent monocytes, and 6 per cent small lymphocytes. At autopsy it appeared that these animals had died of pulmonary infection. The surviv-

ing rabbit did not develop true leukopenia since the count ranged from 19,700 at 24 hours after gassing to 9,500 and 11,050 at 48 to 72 hours, followed by recovery to 28,000 per cmm. at 96 hours. At 48 hours granulocytes fell, but at 72 to 120 hours the count again rose with large numbers of immature forms present. This animal also developed a severe respiratory infection but recovered. Apparently gassing suppressed but did not wholly inhibit granulocyte production, and cells of regenerative type appeared promptly in the circulating blood. These variable effects may reflect variations in the amount absorbed via this route of administration because of unpredictable variations in rate and volume of respiration.

Kethley *et al.*³⁵ found that gassing rabbits with approximately LC_{50} of HN_2 (0.7 to 0.9 mg. per liter for 10 minutes) produced marked leukopenia, 61 per cent of their rabbits dying within 3 days. The animals surviving longer than 3 days showed recovery of the leukocyte count, but half of them died 5 to 6 days after gassing. The cause of death was not discussed by the authors. Lushbaugh²⁶ reported leukopenia in gassed rabbits that subsequently died with pulmonary abscesses and pneumonia after the hemopoietic tissue had recovered and leukocytosis was present.

Other Formed Elements of the Blood. Rabbits dying of HN_2 intoxication do not consistently become anemic, although there has been a slight fall in hematocrit value in most of our animals which was no doubt accentuated by occasional bleeding. Kethley *et al.*³⁵ found no significant changes in the red blood cell count, hemoglobin, and platelet count of gassed rabbits, but they reported that the normal reticulocyte count (4 per cent) fell sharply within 2 days, and we have noted a disappearance of polychromasia. These two facts indicate that at lethal doses erythrocyte production is arrested. The circulating red cells appear unaltered and presumably are capable of living out their life span of 40 to 140 days; since in surviving animals recovery of the hemopoietic tissue occurs within less than 1 week, the hiatus in red cell formation does not generally cause a significant decrease in red cell count or hematocrit.

At LD_{50} dosage reticulocyte formation is depressed up to 4 days, to be followed by the appearance of normoblasts, increased polychromasia, macrocytes, anisocytosis, and poikilocytosis.

It appears from the available data that adult cells in the blood, possibly excepting lymphocytes, are unaffected, the essential fault in intoxicated animals being a failure of cellular replacement in the blood-forming organs, so that those cells having the shorter life span dis-

appear first. The sequence, therefore, is an early fall in lymphocytes and a slow fall in granulocytes and reticulocytes, but only a very slight decrease in red cells and hemoglobin.

The abnormal leukocytes in the peripheral blood are somewhat similar to those seen following the extreme bone marrow stimulation induced by withdrawing leukocytes from the circulating blood by means of the peritoneal exudate technic.³⁰ The occurrence of polycytes and macropolycytes is apparently due to an increase in the rate of maturation of the polymorphonuclear leukocytes, and their presence has been described after various drugs and extracts, x-rays, ultraviolet light, thyroid extract, peritoneal exudates, and infection.³⁷

Following intoxication of the marrow at LD₅₀ dosage, superimposed infection appears to be ineffective as a bone marrow stimulant in preventing the leukopenia from inducing early regenerative activity of the bone marrow; we have observed an early shift to the left in only one of 3 rabbits gassed at a sublethal concentration. Dr. J. MacLeod (personal communication) has been unable to induce leukocyte-containing peritoneal exudates 24 hours after the intravenous injection of HN₂ HCl, and similarly the leukocytosis-producing factor of Menkin given after the intoxication does not prevent the leukopenia or protect the bone marrow of dogs.³⁴ The injection of the leukocytosis-producing factor prior to intoxication in one dog appeared to prevent bone marrow aplasia and leukopenia.³⁴ On this basis, the presence of a pyogenic infection at the time of injection may be expected to modify the effect of HN₂ on the bone marrow. A protective effect of infection on the bone marrow injury induced by benzene has been reported.³⁵

Cameron and Short²¹ demonstrated an increase in sedimentation rate, a decrease in coagulation time, and no change in the red cell fragility in rabbits intoxicated by subcutaneous injection of 3 mg. per kg. of the free base.

Other Species

MacLeod and Rhoads³⁴ observed that *dogs* receiving 2 mg. per kg. of the hydrochloride intravenously developed lymphopenia while the neutrophil count did not fall markedly. At autopsy, however, the bone marrow was aplastic, suggesting that circulating neutrophils are longer-lived in the dog than in the animals reported above. Cameron and Foss¹⁷ observed leukopenia in *goats* following cutaneous application of the free base, and Irwin, Brackenbury, and Young³⁰ have made similar observations in the *monkey*. The demonstration of leukopenia in gassed guinea-pigs, cats, and dogs²⁰ has failed frequently, perhaps because of early death from respiratory injury. In dogs under nembutal anesthesia with the thoracic duct cannulated, the lymph output during

the first 5 hours was at first increased and then decreased, while the lymphocyte content of the lymph decreased. This circumstance resulted in a normal lymphocyte output during this period. One, 2, and 3 days after intoxication, lymph flow was only one-half of normal and the cell count was very much less than normal. Since the decrease in circulating lymphocytes was greater than the decrease in output of lymphocytes, it was concluded that there was an increased disappearance of lymphocytes from the blood in some unexplained fashion.⁴⁰ However, this conclusion should be weighed against the known short life of circulating lymphocytes.⁴¹

TABLE III

Leukocyte and Differential Counts Following 3.0 mg. per kg. of HN₂ Hydrochloride Given Intravenously to a Hen

Days after injection	Total leukocyte count	Differential counts				
		Granulocytes			Agranulocytes	
		Segmented	Basophils	Eosinophils	Lymphocytes	Monocytes
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Control	21,090	71	4	2	23	0
1	49,600	85	1	2	6	6
2	8,200	65	4	8	16	7
3	6,000	50	6	7	33	4
4	2,000	88	7	3	0	2
5	20,550	44	4	3	46	3
6	24,800	41	5	3	48	3
8	25,000	49	3	5	40	3

In the *dog* there is a suggestion that the fall in the granulocytes is slower. Animals given 2 mg. per kg. intravenously died at a time when the granulocyte count was relatively high, although there was almost complete depletion of myeloid tissue at death. This circumstance was attributed to the longer life cycle of the canine granulocyte.³⁴ This delayed granulocytopenia seems confirmed in dogs receiving 1 mg. per kg. intravenously,⁴ but in dogs given 1, 2, and 3 mg. per kg. subcutaneously it is stated that granulocytopenia appears *pari passu* with bone marrow injury and that the granulocyte counts reached low levels in 3 days.⁴⁰

Leukopenia has been induced in *chickens* by sublethal doses of the hydrochloride ranging from 3 to 10 mg. per kg. intravenously. The leukocyte and differential counts on 2 hens receiving 3.0 and 7.5 mg. per kg. are tabulated in Tables III and IV. That these effects were not due to the moderate weight loss suffered by these animals was shown by a control bird that was starved for 6 days without the development of leukopenia. Similar leukotoxic action was observed after injection of H into chickens.

In mice surviving the culmination of hemopoietic injury, regeneration is delayed for several more days. Meanwhile, hemopoietic centers in other organs, notably the liver, show marked evidence of stimulation, so that even in animals dying between the fourth and seventh days some restoration of the leukocyte count may occur prior to death. In surviv-

TABLE IV

Leukocyte and Differential Counts Following 7.5 mg. per kg. of HN₂ Hydrochloride Given Intravenously to a Hen

Days after injection	Total leukocyte count	Differential counts					
		Granulocytes				Agranulocytes	
		Banded	Segmented	Baso- phils	Eosin- ophils	Lymphocytes	Monocytes
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Control	32,650		39	9	2	49	1
1	23,600		96	2	0	1	1
2	1,550*		44	9	13	34	0
3	2,000*	25†	6	19	0	44	6
4	5,100*	20†	0	5	5	35	35
5	8,300	13†	0	18	5	55	9
6	14,500	2	12	6	7	68	5
7	15,850	7	19	4	4	65	1
8	11,000		10	4	4	47	34
11	42,000		43	2	1	53	1
19	21,300		52	11	10	21	6

* Too few cells in the smear to permit a satisfactory differential count, and the figures presented are only approximate.

† Immature cells of the myeloid series not ordinarily seen in the circulating blood.

ing animals the lymphocytes appear to recover before the granulocytes. Histologically, marked hyperplasia in the thymus and lymph nodes parallels this recovery. Granulocytic recovery is spectacular in the blood, the white blood cell count rising from leukopenic to normal, or even supra-normal levels, almost overnight. This phenomenon appears to be related to the outpouring of immature granulocytes, and regenerative activity may persist for 2 to 3 weeks. In mice, a species particularly sensitive to overstimulation of the leukopoietic tissue, hyperplastic foci having the dimensions and intensity of a leukemoid reaction have been observed after an interval of from 3 to 4 weeks.

BLOOD STUDIES AFTER H

A reduction in the number of white cells as well as various biochemical changes in the blood follow parenteral injection or cutaneous application of LD₅₀ or greater doses of H. Blood platelets are markedly decreased only in severely intoxicated animals.⁴² In the early phase the red blood cells apparently are little affected. The reduction in white blood cells involves both granulocytes and lymphocytes; the granulo-

cytes usually are somewhat more sensitive. Leukopenia may be preceded by a nonspecific(?) *leukocytosis* lasting a day or more.³³ In animals *gassed* with H, leukocytosis, leukopenia, and lymphopenia may or may not occur, probably reflecting variations in actual dosage inhaled.

Hemoconcentration indicated by increased red blood cell count, percentage of hemoglobin, and hematocrit value may rapidly follow intoxication. This has been observed in various species after parenteral, topical, and vapor intoxication.⁴³

In surviving animals at a time when the leukocyte count may be returning to normal or above, a progressive anemia may occur with no alteration of the reticulo-endothelial system.⁴³ In rabbits and dogs this anemia frequently resembles the idiopathic or benzol type. The hematocrit reading, percentage of hemoglobin, and red blood cell counts decrease with no change in the color index, mean corpuscular volume, or diameter as measured by the Price-Jones method. Thus it appears that the marrow produces fewer red cells for a time, and that in some instances the cells are immature. The delayed anemia may reflect an initial injury of the erythroblastic tissue which, because of the longer life of the red blood cells, is not evident during the first few days of intoxication. Intestinal hemorrhage also may contribute to the anemia.

HISTOPATHOLOGIC SYSTEMIC EFFECTS

Bone Marrow

One of the most specific features of HN₂ or H intoxication thus far encountered is the depletion of the bone marrow, also described by Zimmerman,⁹ Cameron and Short,²¹ Lushbaugh,^{26,43} and Warren, MacLeod, and Rhoáds.⁴⁴ Sternal and femoral marrow have been examined regularly by us, except in a few instances in which we were limited to the cranial or vertebral marrow, but we have seen no differences in marrow chosen from different sites. Obvious bone marrow depletion does not occur for 24 to 48 hours, irrespective of the dosage, but rats and rabbits receiving lethal doses showed degenerative cellular changes when studied 8 to 30 hours after injection. These changes consist of swelling and dissolution of hemopoietic cells, alterations in their staining reactions, and the presence of enlarged vesicular nuclei and some scattered débris. Mitotic figures can be seen up to 8 hours and are usually absent thereafter until recovery. Agglomerated masses of eosinophilic granules without nuclei may be observed early. In other cells the nuclei are also absent, but scattered throughout the cells are numerous dark-staining chromatin particles suggesting disruption of the nuclei. Suggestive alterations are seen in the megakaryocytes,

consisting of bizarre swellings, but the reticular elements remain unchanged. Warren and Rhoads⁴⁵ have found the injured cells to be very friable, and easily damaged when smeared.

Following this period and varying to some extent with dosage and possibly with the route of administration, depletion of the marrow progresses rapidly to almost complete aplasia in 40 to 90 hours (Figs. 2 and 3). All hematopoietic cells disappear and there is neither shift to the right or left, nor loss or accumulation of specific types. The cells remaining in the marrow are those lining the blood vessels, some reticular cells, fibrocytes, rare macrophages, fat cells, and possibly primitive erythrogenic elements (hemocytoblasts²⁶). There is a tendency for endosteal stem cells to persist, as Dr. C. E. Dunlap (personal communication) has observed in mice injured by x-rays. The megakaryocytes are conspicuous for their bizarre, pyknotic nuclei, and for their persistence in a marrow depleted of its hemopoietic tissue. Chromatin debris is sometimes seen in earlier periods, but it is usually scanty or absent. The marrow is replaced by a loose spongy mass of acidophilic tissue with protein precipitate, fat cells, and dilated sinusoids engorged with erythrocytes.

With large doses animals may die before marrow depletion is complete, but in delayed deaths, as seen in mice, rats, and rabbits at LD₅₀, the bone marrow is invariably hypoplastic and usually aplastic at death. This appears to be true also in dogs.⁴⁵ Irwin, Brackenbury, and Young,³⁹ however, have described only slight degenerative changes in the marrow of monkeys receiving fatal dermal applications of the free base, although the animals became leukopenic. Typical depletion of the marrow follows gassing of mice and rats at LC₅₀.

Animals escaping delayed deaths begin to show regenerative activity throughout the depleted marrow in 4 to 5 days after injection. There is a marked shift to the left as the new stem cells appear,⁴⁵ and mitotic activity becomes evident. During recovery the marrow of rabbits fails to show the normal pseudo-eosinophilia of the granulocytes and Warren and Rhoads⁴⁵ have described abnormal vacuolated cells. These changes may be correlated with the immature cells and "toxic pseudo-eosinophils" described in the circulating blood during the recovery phase. Death may occur when the bone marrow is partially or completely regenerated, but it is our impression that these remote deaths are not due to direct action of HN₂.

Lymphoid Tissue

Involution of the lymphoid tissue is one of the earliest and most striking pathologic effects of both the N and S mustards. The rapidity of this involution was quantitatively determined in rats receiving 3 mg.

per kg. (1.6 LD₅₀) of HN₂ HCl subcutaneously and sacrificed by exsanguination 24, 48, and 72 hours after injection. The weights of the spleens, thymuses, and lymph nodes are shown in Table V. Microscopic observations on these organs were made chiefly in mice and rats, with less complete but analogous observations in rabbits.

TABLE V
Average and Range in Weights of Lymphatic Organs in Rats Intoxicated with HN₂

Hours after injection	No. rats	Average body weight at death	Spleen	Thymus	Cervical lymph nodes
		gm.	mg.	mg.	mg.
Controls	8	178 (169-192)	865 (600-1260)	155 (110-206)	73 (45-100)
24 hours	5	172 (164-184)	541 (375-804)	102 (73-139)	55 (53-56)
48 hours	4	163 (150-178)	446 (337-536)	79 (61-100)	49 (34-70)
72 hours	8	162 (150-185)	233 (168-285)	39 (33-61)	23 (15-30)

Lymph Nodes

The lymph nodes, as in the case of the thymus, show a variable degree of lymphocytic fragmentation, large doses producing intense fragmentation visible in 10 to 48 hours, while small doses cause milder effects. All animals, even at LD₅₀, show loss of follicles and general contraction of the lymph nodes, and on microscopic examination these nodes are seen to be composed of epithelioid cells* and condensed reticular and fibrous elements, with almost complete absence of lymphocytes. As in the case of the spleen and thymus, there is no interference with the mobilization of macrophages and epithelioid cells. Lymph nodes examined up to 3 weeks after intoxication do not show any evidence of hemopoiesis, although lymphocytic hyperplasia was present.

Spleen

This organ likewise exhibits changes which are roughly proportional to dosage. Dosage in excess of LD₅₀ given by any route leads to prompt fragmentation of lymphocytes in the malpighian corpuscles and to a lesser extent in the interstitial tissue of the so-called red pulp (this is best seen in mice), similar to that seen in the lymph nodes and thymus. With time, much chromatin material is found within macrophages in all parts of the spleen. The progressive reduction in the size of the spleen (Fig. 4) results from the disappearance of lymphocytes with atrophy of the corpuscles and loss of cells from the interstitial tissue of the sinus walls. In mice, after smaller doses, megakaryocytes, which are notable in the normal spleen, may increase in number. These often show bizarre, enlarged, pyknotic nuclei, and monolobulated forms are more

* Designated as epithelioid cells because they are large, contain abundant eosinophilic cytoplasm, and appear pavement-like; these cells closely resemble macrophages and may be the same cells, differing only in the absence of readily discernible phagocytized material.

common than multilobulated cells. At 48 hours the disappearance of lymphocytes from the corpuscles and from the red pulp gives the organ a somewhat fibrous appearance. At the periphery of the malpighian corpuscles epithelioid cells or fibroblasts are seen, and occasionally small foci composed of similar cells may be found apart from the corpuscles.

During the first 2 days of intoxication, intact polymorphonuclear leukocytes or banded forms, and occasionally eosinophilic myelocytes, may be seen in a spleen in which the lymphocytes have undergone intense fragmentation or sharp reduction in number. The ultimate stage in the regression of the spleen is reached at 70 to 100 hours, at which time the neutrophils also have disappeared. Necrotic changes in the corpuscles may no longer be visible, although nuclear debris may still be found in occasional macrophages. In some animals a small number of lymphocytes may persist, but the malpighian corpuscles are composed largely of reticular cells.

At LD_{50} doses or less, the spleen may appear fairly normal in the first 48 hours, except for a variable increase in megakaryocytes, but thereafter it undergoes progressive contraction. Polymorphonuclear leukocytes are progressively reduced in number from about 40 hours onward until recovery begins. How this is accomplished is not clear, for no increase in chromatin debris is visible. After 5 or 6 days the animals that have survived at LD_{50} frequently show excessive myelopoiesis; the corpuscles thereafter enlarge, and contain a rich mixture of lymphocytes with reticular cells, although the former are overwhelmingly preponderant. In the red pulp, clusters or cords of primitive hemopoietic cells reappear and polymorphonuclear leukocytes are found in large numbers around these areas. Plasma cells are usually conspicuous in these foci. There is little evidence of hemolysis, as indicated by storage of hemosiderin in the macrophages, and when present there is a question whether this pigment represents coincident or antecedent hemolysis.

Thymus

Doses considerably in excess of LD_{50} regularly produce marked thymic necrosis which is best seen from 12 to 48 hours after administration. At LD_{50} or less, the thymic effects are more gradual in their evolution, especially in rats and rabbits as compared to mice, but the organ contracts (Fig. 5) with more or less complete replacement of the cortex by reticular or epithelioid cells. After gassing or oral administration, in the range of LD_{50} , the effects on the thymus are less striking than after parenteral administration, and usually consist of moderate atrophy with little lymphocytic fragmentation.

The lymphocytic fragmentation observed after large doses generally occurs by karyorrhexis; karyolysis or fading out of the nuclear structure has been less frequently observed, and is most evident in animals that do not show much karyorrhexis. Lymphocytic fragmentation progresses to an advanced stage within 24 hours. The nuclear debris is usually free in early stages, but macrophages appear later and most of the material is finally engulfed within the macrophages or disposed of by other means.

When the thymus undergoes involution, the cortex shrinks somewhat more rapidly than the medulla, and during the period of cortical contraction the lymphocytes may appear more numerous in the medulla than in the cortex, thus tending to reverse the normal pattern. In surviving animals this reversal may be corrected, the cortex reassuming its normal lymphocytic appearance, while the medulla becomes conspicuous for its reticular cells and epithelial elements. The profoundly involuted thymus, found 72 to 96 hours after LD_{50} , is small, fibrous, and stringy in appearance, while in other animals surviving LD_{50} or slightly less, the thymus may exhibit hyperplastic changes at 90 hours or later, and in many animals examined after 1 week the organ appears to have undergone an increase in size.

Changes similar to those observed by us in the lymphatic tissues have been recorded by Zimmerman,⁹ Cameron and Short,²¹ Lushbaugh,²⁶ and Irwin, Brackenbury, and Young.³⁹ The early and graded injury of lymphopoietic tissue, and its rapid recovery after LD_{50} , is correlated with changes in the number of circulating lymphocytes. Lymphopenia occurs precipitously during the first 24 hours and may become extreme if intoxication is fatal. At lower doses the count may show a plateau after 24 hours and begin to rise on the fourth to fifth day. There is no correlation between lymphopenia and the ensuing granulopenia, in that at low doses lymphocytes show an immediate moderate but reversible fall, while on the third to fourth days a severe granulopenia may appear. Also, lymphocytes, although affected earlier, are not necessarily more sensitive than the bone marrow cells as far as the ultimate severity or duration of effect is concerned.

Intestinal Tract

Clinical disturbances and gross alterations in the intestinal tract of intoxicated mice, rats, and rabbits, and histologic preparations of rolled-up segments of the entire intestines in serially sacrificed mice, rats, and rabbits receiving 1 to 2 LD_{50} doses demonstrate consistently the presence of extensive intestinal injury. The lesion starts just below the pylorus and extends the entire length of the small intestine, with the

ileum most severely involved. The stomach and Brunner's glands appear unaffected, but microscopic focal epithelial lesions may be found in the first part of the colon.

The early process is degenerative and inflammatory (Fig. 6-B), involving most of the mucosa, with epithelial injury and erosion being marked. Within 24 to 48 hours after injection the villi become edematous and the lining cells appear swollen and may be distended with clear vacuoles, leading finally to cellular rupture and desquamation. The mucosa may show edema and fibrin beneath the lamina propria, hyperemia, dilation of the lymphatics, and a variable inflammatory reaction consisting largely of polymorphonuclear leukocytes and eosinophils. Necrotizing changes in the interstitial cells, including the lymphocytes, are not conspicuous. Rats given trypan blue intravenously or intraperitoneally exhibit selective deposition in the mucosa of the small intestine after 48 hours.

At 72 to 96 hours, when the lesion is most severe, a mixture of extensive injury, metaplasia, and regenerative activity of the epithelium is seen (Figs. 6-C and 6-D). The crypts of Lieberkühn appear as cyst-like spaces lined by flat, elongated cells, and occasionally contain an accumulation of polymorphonuclear leukocytes, nuclear debris, and desquamated cells. In other areas the crypts may be lined by hypertrophic, hyperchromatic cells. The mucosal surface may be denuded, or show patchy areas of metaplasia, or epithelial hypertrophy and hyperplasia with vesicular swelling of the nuclei. The regenerating cells are flattened and occasionally show surface spines. They usually contain hyperchromatic nuclei composed of large chromatin masses. Mitotic figures are common. Goblet cells show increased mucus formation, especially in the colon. The maximal epithelial alterations and sloughing coincide in time with the development of fluid distention and mucous diarrhea. In later stages a slight fibrous reaction may lead to deposits of connective tissue in the villi which tend to become shorter, broader, and stubby. Rats show these intestinal changes more strikingly, but they are also apparent in mice and rabbits.

The significance of the functional and pathologic changes in the intestinal tract in relation to death of the animals, whether by loss of fluid and alkali, by increased permeability of the intestinal wall to injurious substances, or by the lowering of the local defense mechanism against the invasion of bacteria, raises problems treated elsewhere.⁴

Respiratory System

Animals receiving 1 to 3 LD₅₀ parenterally do not show any consistent or severe injury to the upper or lower respiratory tract. Guinea-

pigs receiving large doses subcutaneously are reported to develop pulmonary edema, but not after intraperitoneal injection.¹² Cameron and Foss¹⁷ have described, in goats, edema and congestion of the lungs, and gross congestion and hemorrhages in the trachea and larynx after subcutaneous injection of large doses. Pulmonary edema has been seen occasionally by us in rats receiving the hydrochloride intravenously, especially in those subjected to an abdominal operative procedure at the time of injection, but the phenomenon has not been examined in detail.

Circulatory System

Animals injected parenterally do not show any consistent injury to the heart or blood vessels.

Excretory System

None of our animals has shown any specific injury to the kidneys or urinary bladder. Lushbaugh²⁶ has found infiltration and parenchymatous degeneration, *e.g.*, nuclear fading, pyknosis, and cytoplasmic sloughing, in the proximal and distal convoluted and collecting renal tubules, especially in mice and rats, but such changes as we have seen do not exceed those observed in fasted nonintoxicated animals.

Reproductive System

Some sections of mouse testes have suggested maturation arrest in spermatogenesis, but to evaluate injury to the generative organs, better controlled stock than we possessed at the time of observation is required. The picture is complicated by the severe weight loss, which is known to diminish pituitary activity.¹⁴ We have noted that following an LD₅₀ of the hydrochloride, surviving female mice do not become sterile, and that chickens can resume laying eggs.

Endocrine System

The pituitary gland was not studied. The thyroid and pancreas are not affected, but in the rat the adrenals are increased 60 per cent in weight and cortical hypertrophy and depletion of lipid are noted. The adrenal hypertrophy may be comparable to that described by Selye⁴⁶ in rats subjected to a variety of noxious stimuli.

Liver

No impressive changes have been seen in the livers of any of the species studied. Glycogen depletion is fairly common, especially in animals receiving large doses, and slight fatty infiltration is noted 3 to 4 days after injection, especially in mice. Even after oral administra-

tion (when lethal doses are absorbed partly into the hepatic-portal system to be routed directly into the liver), or after intrasplenic injection (when the material is circulated directly through the liver) there is no obvious injury of the liver parenchyma. Zimmerman,⁹ however, reported focal necroses in the livers of cats but not rats after the oral administration of the hydrochloride.

Lushbaugh²⁰ also has described fatty infiltration in the mouse liver 4 to 6 days after gassing. This increased fat may be attributable to progressive anorexia and fasting, since Hodge *et al.*¹² have shown in fasted mice that the total liver lipid increases to 2 to 3-fold in the first day and decreases to normal on the third to fourth days; coincidentally, however, the liver rapidly decreases in size, so that the percentage of fat in the liver is usually above normal. We have observed also an increase in histologically demonstrable fat in the livers of starved control mice.

COMPARISON WITH EFFECTS OF H

For producing visible systemic injury in rats, H is superior at LD₅₀ doses to the β -chloroethyl vesicants (HN₁, HN₂, and HN₃), when administered intravenously or subcutaneously. In a comparative study of the delayed systemic action of H, HN₁, HN₂, and HN₃, rats were exposed to these compounds via cutaneous application, gassing, intravenous and subcutaneous injection at dosage in the range of the LD₅₀ or LC₅₀, and about 0.5 LD₅₀ or 0.5 LC₅₀. Weight loss, leukopenia, lymphoid atrophy, myeloid changes, and enteritis were measured or sought in each animal. Almost all were sacrificed at 72 or 96 hours after exposure to, or administration of, the agents; a few were included at 48 and 120 hours after exposure. Wide variations occur between the intravenous and subcutaneous routes when compared with the effects following cutaneous application or gassing. Text-Figure 3 shows the intensity of total systemic injury and of the individual lesions following the levels of dosage used. After gassing and percutaneous application, the systemic effects of HN compounds, at both levels of dosage, were more severe than after H, with the single exception that HN₁ was less myelotoxic than the other three compounds. On the basis of dosage required, HN₃ was the most efficient in producing delayed systemic effects. Intravenous and subcutaneous injection of H (in propylene glycol) reverses the relationship to the HN compounds; at the LD₅₀ dosage it was most consistent and toxic in its action, judging by the loss of weight, leukopenia, extent of lymphoid atrophy, bone marrow injury, and enteritis. HN₃ was again the most efficient of the nitrogen mustards; HN₁ was the least leukotoxic at the dosage employed. At the sub-LD₅₀ or sub-LC₅₀ dosage these differences among

the compounds were maintained with all routes of administration but were not so striking.

COMPLICATIONS AND VARIATIONS IN INTOXICATED ANIMALS

Some of the unusual effects and complications observed after administration of the HN₂ are worthy of record. These include occurrence of reactivated or superimposed infection and its relation to remote deaths, the appearance of extramedullary myeloid metaplasia in mice during recovery, and a hemolytic episode in a single rabbit.

Infection

The frequent presence of obvious infection (which must heighten our suspicions as to the existence of occult infection) has been alluded to several times. In those occasional deaths occurring 7 days or longer after the parenteral injection of the hydrochloride ("remote deaths") it is necessary to rule out infection before a direct but remote toxic action can be considered. Early in our work such remote deaths were rare in mice, but about 2 months later mouse "typhoid" appeared in our colony, following which the number of remote deaths in intoxicated animals increased sharply. In many instances multiple necroses of the liver were present in these animals. Unfortunately, bacteriologic examination could not be made at the time.

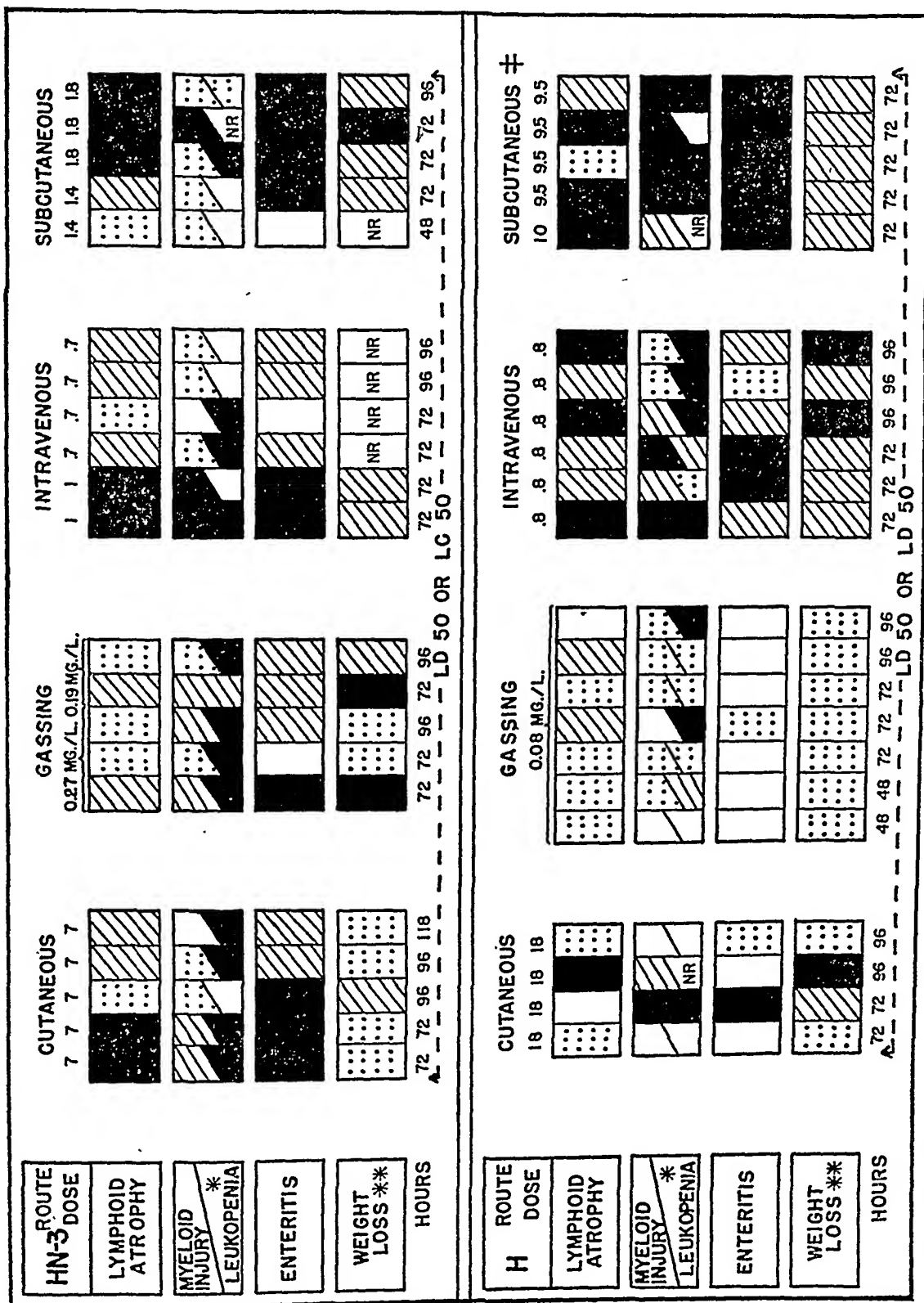
Complete pathologic studies were available in a few rabbits dying 4 days or longer after intravenous injection, at which time their leukocyte counts showed recovery. The pathologic diagnoses included pneumonia with septicemia, infected thrombi and abscesses in the lungs, heart, liver, kidneys, and gonads, necrotizing bronchopneumonia, and reactivation of coccidiosis in the intestine. The last is a pathologic rarity, but is described also after exposure of rabbits to x-rays⁴⁷ and mustard(H).⁷

It is not surprising to find secondary infection much more commonly following gassing, since the combination of severe local respiratory injury, systemic intoxication, and leukopenia present circumstances favorable to the development and spread of infection. Such infections are usually in the respiratory tract, and are particularly common in rabbits as compared to mice and rats.

All remote deaths, however, need not be attributed to infection alone. The marked weight loss, well within the range of fatal weight loss due to simple starvation, suggests that starvation may be an important and perhaps decisive factor in some cases. It is not clear whether starvation is due to debility produced by the primary intoxication which prevents the animal from eating, to changes in the endocrine and nerv-

CUTANEOUS		GASSING		INTRAVENOUS		SUBCUTANEOUS	
HN-1 ROUTE DOSE	16.5 16.5 17 17 17 17 17	0.16 MG./L.	50 50 72 72 96 96 120	50 50 72 72 96 96 120	72 72 72 72 72 72 72	70 72 72 80 96	
LYMPHOID ATROPHY							
MYELOID INJURY * LEUKOPENIA							
ENTERITIS							
WEIGHT LOSS**							
HOURS	72 72 72 96 96 120 120	50 50 72 72 96 96 120	50 50 72 72 96 96 120	50 50 72 72 96 96 120	72 72 72 72 72 72 72	70 72 72 80 96	

CUTANEOUS		GASSING		INTRAVENOUS		SUBCUTANEOUS	
HN-2 ROUTE DOSE	26 26 26 26	0.41 MG./L.	72 72 96 120	72 72 72 72 72 72 72	72 72 72 72 72 72 72	72 72 72 96	
LYMPHOID ATROPHY							
MYELOID INJURY * LEUKOPENIA							
ENTERITIS							
WEIGHT LOSS**							
HOURS	48 72 72 96	72 72 96 120	72 72 72 72 72 72 72	72 72 72 72 72 72 72	72 72 72 72 72 72 72	72 72 72 96	



* 4,000 to 2,000 white blood cells per cmm. graded as mild; 2,000 to 1,000 white blood cells per cmm. graded as moderate; 1,000 or less white blood cells per cmm. graded as severe.

** Weight loss of 2 to 9 per cent regarded as mild; 10 to 20 per cent regarded as moderate; 20 to 30 per cent regarded as severe.

‡ H in propylene glycol.

ous systems, or digestive tract, or to undetected infection which adds to and complicates other debilitating factors.

Extramedullary Myeloid Metaplasia

Mice surviving LD₅₀ doses show active hemopoiesis in the liver and spleen, this process sometimes assuming the intensity of a leukemoid reaction, and being markedly developed at 1 month. We have not followed our surviving mice beyond 1 month in systematic pathologic studies, but some mice surviving an LD₅₀ dose administered subcutaneously were sacrificed after 1 to 5 months and showed variable degrees of extramedullary myelopoiesis. In some animals regression had occurred. Rabbits surviving longer than 7 days after the injection of the hydrochloride have shown evidence of active hemopoiesis in the spleen.

Similar extramedullary hemopoiesis, presumably of a compensatory nature, has been described in mice and rabbits after a wide variety of procedures, including repeated bleeding, the injection of various poisons such as saponin, pyrogallol, phenylhydrazine, and benzol, and the injection of live or dead bacteria.⁴⁸ It is unlikely, therefore, that the appearance of myeloid metaplasia after HN₂ represents a specific effect, or that it necessarily presages the possible later development of leukemia.

Hemolytic Episode

Of a large group, a single rabbit receiving 2 mg. per kg. of HN₂ hydrochloride developed hemolytic anemia, as evidenced by a marked fall in the hematocrit value and red cell counts and an icteric plasma. Death occurred with bronchopneumonia 9 days after injection. This recalls isolated hemolytic episodes associated with various drugs, *e.g.*, sulfonamide compounds.

SPECIES DIFFERENCES

While the data are not numerous, all mammals studied appear to possess about the same general degree of susceptibility to the parenteral administration of HN₂. The gassing LC₅₀ for different species spreads over a wider range, but this may be accounted for in part by the greater susceptibility of the respiratory tract in large animals, variations in respiratory pattern, and differences in local fixation and absorption. The rat appears to be the least resistant. It is only 70 per cent as resistant as the mouse by any route of administration including gassing. Newborn rats possess no special resistance to subcutaneous injections of HN₂.

Chickens and pigeons possess considerable resistance to the in-

travenous injection of the hydrochloride. The LC_{50} in chickens is about 10 mg. per kg. At this dose chickens may show some weakness and moderate weight loss which is slowly recovered, or more severe weakness with incoordination which usually ends fatally within a few hours. The picture of delayed death which has been described in mammals is not found in the chicken. Pigeons also may survive 10 mg. per kg. intravenously, but neurologic symptoms are more marked, weight loss is considerable, and the birds may die several weeks after injection. The LD_{50} is less than 10 mg. per kg. in pigeons. The resistance of chickens is the more notable since severe leukopenia occurs after doses of 3 to 10 mg. per kg. intravenously. At none of the tested doses did diarrhea or enteritis appear. A similar degree of resistance was not found in a single experiment using H in chickens, but the cause of death was not determined in this experiment nor were post-mortem studies included.

A COMPARISON OF THE EFFECTS OF BENZENE AND RADIATION WITH THOSE OF HN_2

Since HN_2 appears to be highly injurious to the bone marrow, it is of interest to compare its effects with those of two well known leukopenic agents, benzene and radiation (x-ray, radium, and neutrons).

Benzene

A single lethal dose will produce an early death, with neurologic or hemorrhagic manifestations, but without leukopenia, while a single sub-lethal dose may cause a transient stimulation of the bone marrow. However, repeated doses of 1 to 2 cc. per kg. daily subcutaneously produce a progressive leukopenia which may approach an almost complete absence of leukocytes in 4 to 10 days.⁴⁸⁻⁵⁰ Simultaneously, the lymphoid tissue shows fragmentation and involution, followed by aplasia of the bone marrow. The marrow injury consists of visible pyknosis and fragmentation of all hemopoietic cells. If the injections are continued after severe leukopenia is present, the animals will die. The cause of death is not clear, although it is known that infection plays an important rôle. If benzene is stopped when the leukopenia is severe, recovery may be rapid, and ordinarily there are no obvious sequelae. Aside from an inconstant weight loss, no notable symptoms are present, even during the leukopenia. At autopsy, the atrophic lymphatic tissue and marrow, and occasional degenerative changes in the kidneys and liver are the only evident lesions, the intestinal tract being normal. The leukopenia prevents the phagocytosis of invading bacteria⁵¹ and, since antibody formation is also diminished,⁵² benzene-

treated animals are markedly susceptible to infection.⁵³ With the exception of the presence of leukopenia, lymphoid and bone marrow injury, benzene poisoning is thus seen to differ in many respects from HN₂ intoxication.

Radiation

The destructive action and the extensive and prolonged alterations induced in tissues by the direct application of x-rays are well-known, and recently have been the subject of a series of reviews by Warren and his colleagues.⁵⁴ Animals exposed to sufficiently large doses of "total body" radiations survive 4 to 7 days, and then succumb to "general intoxication."⁵⁵⁻⁵⁸ Although variation exists among species, the following generalizations may be made: There is a short latent period following which anorexia, progressive weight loss, diarrhea, and extreme prostration lead to death. Lymphocytes and then granulocytes disappear from the circulating blood within 3 to 4 days, but anemia develops slowly. No biochemical changes in the blood incompatible with life have been found,⁵⁴ although generally there is evidence of an increase in protein catabolism.⁵⁶ At autopsy the lymphatic organs are atrophic, the intestines may be ulcerated, hemorrhagic, and distended, and the bone marrow is aplastic. Histologically, the lymphatic tissue appears to be most sensitive, and early necrosis and fragmentation of the lymphocytes and involution of the spleen and thymus are found. The marrow shows early damage by loss of staining properties and lysis, or by pyknosis and fragmentation of nuclei, but aplasia is not complete for 2 to 3 days. Extensive ulcerations and epithelial changes occur in the intestine. These effects are due to the direct action of the x-rays,^{54,59} although trauma and secondary infection may be contributing factors for the intestinal lesions.⁵⁴ Remote deaths following x-ray irradiation are often related to infection, which may be in part attributed to leukopenia and diminished antibody formation⁶⁰ and in part to the dissolution of the epithelial defense barrier represented by the intestinal mucosa, which is extensively or completely destroyed.⁶¹

Lawrence and Tennant⁶² have reported a study of the effects of x-rays and neutrons on mice, which furnishes an interesting parallel to our observations on HN₂ intoxication. Large doses induced death in 4 to 7 days, but at smaller doses deaths occurred up to 49 days. Females recovering were sterile, while males showed temporary sterility. Mice autopsied before 7 days showed lymphatic and bone marrow injury, and characteristic intestinal lesions, whereas animals dying after this period suffered continued weight loss, but at death various stages of bone marrow recovery were found. Blood cultures of mice dying before 4 days were sterile, but between 4 to 7 days they were occa-

sionally positive. Cultures obtained from mice dying after this period always contained bacteria, whereas they were sterile in sacrificed recovering mice. Lawrence and Tennant concluded that death following large doses of x-rays or neutrons is not due to infection but is related to "marked destructive changes in the various viscera mentioned above, giving rise to a toxemia from tissue breakdown products." As the dose is decreased, mice live longer, bacterial invasion is more likely, and infection comes to play a more important rôle. Chrom⁶¹ believed that x-rayed mice die through a combination of intoxication, injury to the reticulo-endothelial system, and absence of defense against bacteria, so that finally fatal bacterial invasion occurs. Of interest in this connection is the demonstration by Osgood⁶³ that x-rays do not cause immediate cell death in cultures of bone marrow but interfere with mitotic activity, and the demonstration by Warren and Whipple⁶⁴ that chickens and pigeons are relatively resistant to radiation.

In general, then, lethal doses of x-ray appear to produce an extraordinarily close parallel to HN₂ intoxication, but this parallelism for the moment can be regarded only as superficial, since in neither case has the fundamental mode of injury or the ultimate cause of death been determined.

SUMMARY AND CONCLUSIONS

1. The nitrogen and sulfur mustards are readily absorbed from the skin and mucosal surfaces, inducing injury to the lymphatic tissue, spleen, bone marrow, and the epithelium of the small intestine, and delayed death 3 to 6 days later.

2. The sequence of events at LD₅₀ doses consists of a relatively asymptomatic latent period of 1 to 2 days. During this time lymphatic injury is abrupt, the thymus, spleen, and lymph nodes involute rapidly, showing karyorrhexis, some karyolysis of the lymphocytes and depletion of these cells, phagocytosis of the debris, and a persistence and proliferation of epithelioid cells; at the same time there is a rapid reduction of the lymphocyte count in the peripheral blood. The hemopoietic cells of the bone marrow show injury, evidenced by changes in the staining reaction, vesiculation and fragmentation of nuclei, karyolysis, and nuclear alterations in the megakaryocytes. The epithelium of the small intestine shows vacuolization and nuclear swelling.

3. Following this period, anorexia, weight loss, and mucoid diarrhea ensue, and finally prostration and death occur in 72 to 144 hours. During this time lymphatic atrophy persists. The hemopoietic cells of the marrow disappear uniformly and the marrow becomes aplastic, consisting of dilated sinusoids, fat cells, and protein-rich fluid with a scattering of surviving cells, including megakaryocytes, reticular

and endosteal cells. The peripheral blood is severely leukopenic, due to a progressive fall in granulocytes. The red count falls slightly, but reticulocytes disappear, and it is evident that the production of hemopoietic cells has ceased. The small intestine is distended with fluid; and gastric stasis, possibly attributable to pyloric spasm, is consistently present in small animals. From the pylorus to the cecum the small intestine shows inflammatory and degenerative changes, with hyperemia, edema of the villi, sloughing of the epithelium, and metaplastic changes in the persisting and regenerating epithelium. Significant pathologic changes have not been observed in other tissues. The cause of these delayed deaths has not been determined.

4. Animals surviving LD_{50} doses gradually recover weight and show restoration of bone marrow and lymphoid tissue and a return of leukocytes in the peripheral blood. The lymphocytes return rapidly, the granulocytes more slowly; and, as seen in the rabbit, recovery is characterized by a shift to the left, with the appearance of pseudo-eosinophilic polymorphonuclear leukocytes containing basophilic granulations, macropolycytes, agranular polycytes, basophils, and occasional abnormal red cells. The diarrhea subsides, and the intestinal epithelium is restored to normal.

5. Other animals show similar restoration of the bone marrow and leukocyte count, but continue to lose weight, with or without obvious secondary infection, and die at a remote period. These deaths do not appear to be directly related to the primary effects of HN_2 .

6. Large doses parenterally induce rapidly appearing neurologic symptoms: convulsions, depression, incoordination, irritability, tremors, weakness, dyspnea, and parasympathomimetic activity. Death occurs in 1 to 40 hours, varying with dosage.

7. In mice, rats, and rabbits gassed at LC_{50} concentrations most of the vapor is removed in the upper respiratory tract and a considerable portion is absorbed to induce systemic intoxication, as evidenced by the usual hematologic and morphologic changes, and the occurrence of delayed deaths. Upper respiratory injury is severe and prolonged, and random deaths with continued weight loss and respiratory infection may occur at long periods after gassing. Large animals (dogs, cats, goats) suffer more severe pulmonary injury and die somewhat earlier at concentrations of the vapor possibly insufficient to induce lethal systemic intoxication.

8. When the hydrochloride is given orally, injury of the duodenal and jejunal mucosa with ulceration, hemorrhage, and sometimes perforation occurs; squamous metaplasia of the intestinal epithelium is a feature of healing. The presence of food in the gastrointestinal tract

appears to exert a local protective action. The compound may be absorbed from the intestinal tract to induce systemic intoxication.

9. The mammals which have been studied show no great differences in susceptibility, but chickens are relatively resistant to the intravenous injection of the hydrochloride, succumbing only to doses inducing an early neurologic effect (*i.e.*, they do not show the phenomenon of delayed death characteristic of mammals). Transient leukopenia occurs in the surviving birds.

10. For producing visible systemic injury in rats, H is superior at LD₅₀ doses to the β -chloroethyl vesicants (HN₁, HN₂, and HN₃), when administered intravenously or subcutaneously.

11. In a comparative study of the delayed systemic action of H, HN₁, HN₂, and HN₃, rats were exposed to these compounds via cutaneous application, gassing, intravenous and subcutaneous injection at dosage in the range of the LD₅₀ or LC₅₀, and about 0.5 LD₅₀ or LC₅₀.

a. Wide variations occur between the effects obtained by the *intravenous* and *subcutaneous* routes and those following cutaneous application or gassing.

b. After *gassing* and *percutaneous* application the systemic effects of HN compounds, at both levels of dosage, were more severe than after H, with the single exception that HN₁ was less myelotoxic than the other three compounds. On the basis of dosage required, HN₃ was the most efficient in producing the delayed systemic effects.

c. *Intravenous* and *subcutaneous* injection of H (in propylene glycol) reverses the relationship to the HN compounds; at the LD₅₀ dosage, it was most consistent and toxic in its action judging by the loss of weight, leukopenia, extent of lymphoid atrophy, bone marrow injury, and enteritis. HN₃ was again the most efficient of the nitrogen mustards; HN₁ was the least leukotoxic at the dosage employed.

12. Comparison of the delayed systemic effect of nitrogen and sulfur mustards with other leukopenic agents (benzene and x-rays) shows them to be remarkably similar to x-rays (including the enterotoxic action), whereas parallelism to benzene is less evident.

For an interesting comprehensive and quantitative study of the effects of single intravenous injections of the same nitrogen and sulfur mustards at doses approximately equal to our LD₅₀ dosage, the reader is referred to the paper by Kindred, J. E., *Histologic changes occurring in the hemopoietic organs of albino rats after single injections of 2-chloroethyl vesicants*. *Arch. Path.*, 1947, 43, 253-295, which appeared after our paper had been submitted for publication. Kindred's general observations are in essential agreement with ours.

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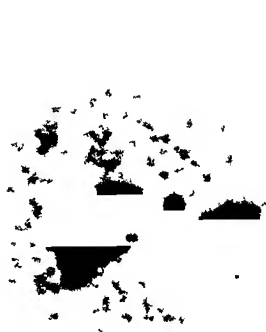
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE I

FIG. 1. Polymorphonuclear leukocytes which may occur in rabbit blood during the recovery phase. These cells first appear from 4 to 5 days after administration of 0.5 to 3.0 mg. per kg. of HN₂ hydrochloride and may persist for 2 weeks thereafter.

A. Macropolycyte: nucleus in mitosis, basophilic granulations. B. Pseudo-eosinophilic polymorphonuclear leukocytes: cell on the right shows an excessively segmented nucleus. C. Pseudo-eosinophilic polymorphonuclear leukocytes: basophilic granulations. (This is a constant and the most common form of abnormal cells.) D. Lymphocyte and an agranular pseudo-eosinophilic polymorphonuclear leukocyte.



1-A



1-B



1-C



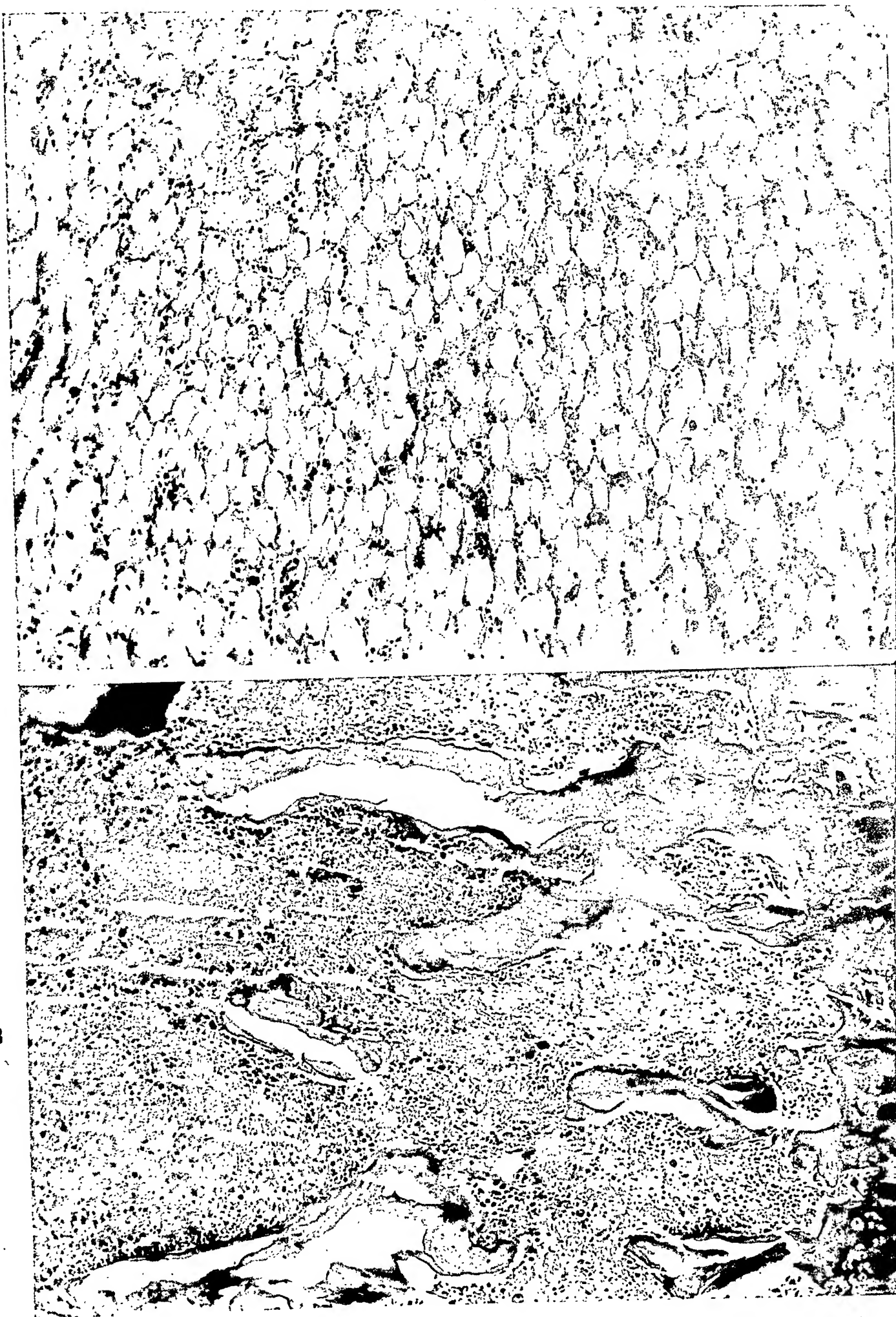
1-D

Graef, Karnofsky, Jager, Krichesky, and Smith

Effects of Nitrogen and Sulfur Mustards

PLATE 2

FIGS. 2 and 3. Femoral (Fig. 2) and sternal (Fig. 3) bone marrow of a rabbit given 3.0 mg. per kg. of HN_2HCl intravenously and sacrificed 70 hours later. The photomicrographs illustrate the severe depletion of cells and the persistence of fat and an irregular deposit of protein-containing fluid in the interstitium. Figure 2, $\times 170$; Figure 3, $\times 130$.



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Effects of Nitrogen and Sulfur Mustards

PLATE 3

FIG. 4. Low-power photomicrograph illustrating splenic contraction in a rat 72 hours after gassing for 10 minutes at 0.4 mg. per liter. The follicles are about one-half their usual size, and the red pulp is represented by fibrous strands and sinuses engorged with erythrocytes. Hematoxylin and eosin stain. $\times 30$.

FIG. 5. Low-power photomicrograph illustrating thymic involution in a mouse 70 hours after receiving 16 mg. per kg. of HN₂ subcutaneously. Of note are the great reduction in lymphocytes of the cortex and the replacement by large epithelioid cells. Lymphocytes persist in the medulla. The pattern suggests a reversal of the normal corticomedullary composition. Hematoxylin and eosin stain. $\times 30$.



4



5

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PLATE 4

FIG. 6. Photomicrographs of sections of the jejunum of rats.

A ($\times 150$) illustrates the villi and normal appearance of the epithelium in an untreated animal (apparently free from paratyphoid infection or parasitic enteritis). B and D illustrate the enterotoxic effect of HN₂ hydrochloride at 48 hours (3 mg. per kg. subcutaneously) and C, at 96 hours (2 mg. per kg. intravenously). All sections were stained with hematoxylin and eosin.

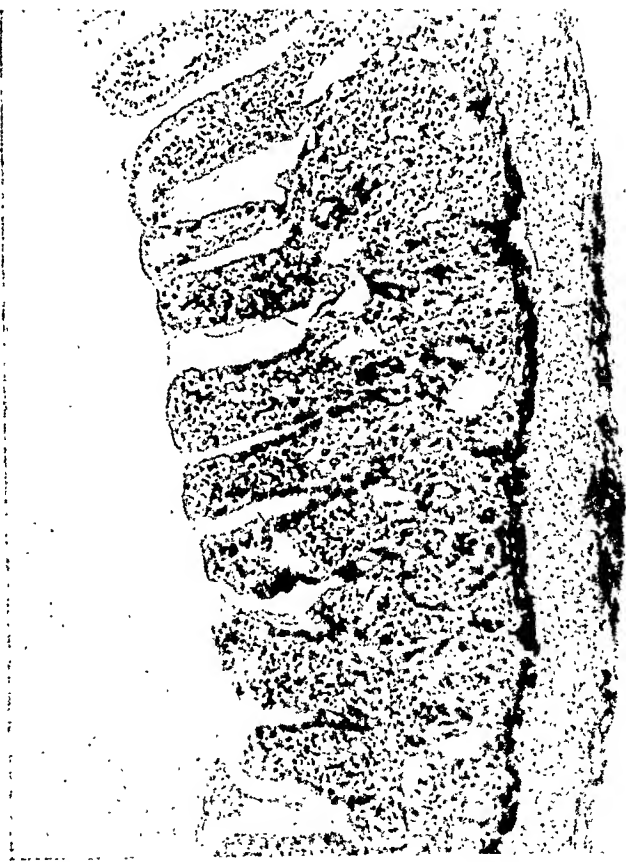
In B, the villi are broader than normal and shorter. Subepithelial edema has lifted the lining cells of the villi. Deep in the mucosa, dilated lymphatics may be seen. These often contain precipitated protein-rich fluid. There is an increase of cells, chiefly eosinophilic leukocytes and some polynuclear leukocytes. $\times 100$.

In C, a later stage is depicted. Of note are the striking vesiculation of the cytoplasm of the surface cells and evidence of desquamation. The villi are short and abut on each other. In the crypts of Lieberkühn, squamous metaplasia has taken place and the cells of Paneth are no longer seen. The cellular reaction in the interstitium is not marked or in excess of normal findings, as is often the case. $\times 250$.

In D, the villi are short, stubby, and the mucous epithelium is partially replaced by hypertrophic and hyperchromatic cells with large nuclei. In places, the epithelium is low-columnar; in others, it is of a squamous character. In one crypt is a large plug of detritus including nuclear debris and some polynuclear leukocytes. $\times 250$.



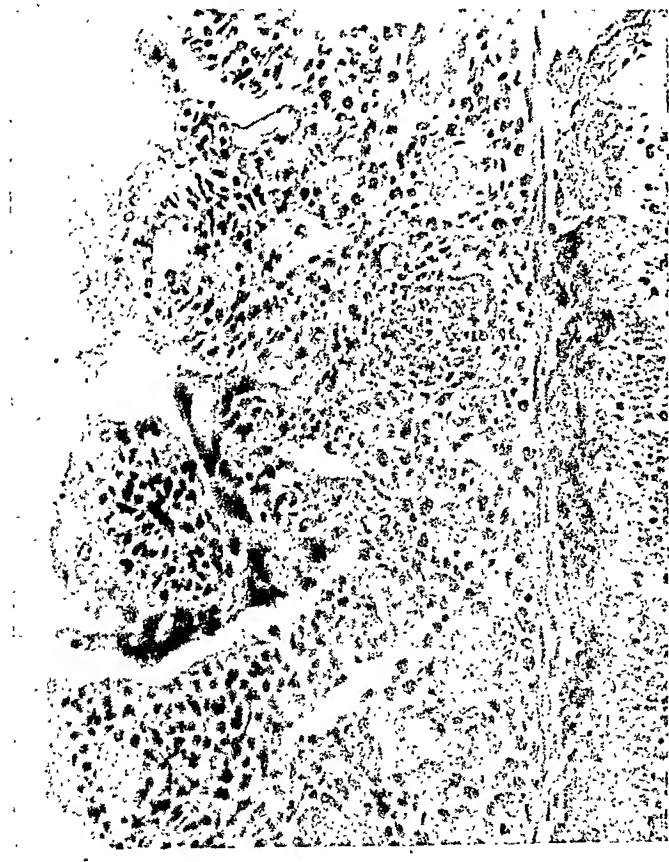
G-A



G-B



G-C



G-D

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Effects of Nitrogen and Sulfur Mustards

SOME ASPECTS OF THE EVOLUTION OF SILICOTIC LESIONS*

I. COSTERO, M.D.

(From the Department of Pathology, University of Mexico School of Medicine,
and the Instituto Nacional de Cardiología, Mexico, D.F.)

The microscopic appearance of silicotic lesions is well known today and may be found described in the standard textbooks on pathology. These descriptions are based on the classical staining technics with aniline dyes. I have studied 35 cases of pulmonary silicosis with the silver impregnation technics of Río-Hortega and have obtained a number of new or little known structural details which may contribute to a better understanding of the evolution of the silicotic nodule and which explain the presence of those other non-nodular lesions that are constantly observed in the lungs of patients with silicosis.

Silicotic lesions are not due exclusively to the direct effect of silica dust inhaled into the alveoli but are influenced also by secondary infections. However, these infections, especially tuberculosis, are present so constantly with silicosis that it becomes difficult to trace a sharp line between lesions which are purely silicotic and those which are mixed; therefore, they are being described together.

TECHNICAL METHODS

The staining methods employed in this report are described in detail in the publications of P. del Río-Hortega, which are listed in the bibliography.† The following is a summary of those methods which gave the best results in staining silicotic nodules.

Río-Hortega's Method for Precollagen Fibers

1. Fix in 10% aqueous solution of formalin (indefinite time).
2. Cut sections on the freezing microtome.
3. Wash in distilled water (15 minutes).
4. Immerse in a recently prepared 5% aqueous solution of potassium permanganate; each section must remain in this solution a different period of time, i.e., 15, 30, and 60 seconds, and 2, 4, 8, and 16 minutes; thus seven sections will be necessary.
5. Wash rapidly in distilled water (5 to 15 seconds).
6. Remove the potassium permanganate retained in the sections with a 5% aqueous solution of oxalic acid (15 seconds).
7. Wash rapidly in distilled water (5 to 15 seconds).
8. Wash in ammoniacal water: distilled water, 50 cc.; ammonium hydroxide, 1 drop (5 to 15 seconds).
9. The seven sections which have followed all the steps, and another two not

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† These publications may be obtained in the Institución Cultural Española, Bernardo de Irigoyen 672, Buenos Aires, República Argentina.

previously treated with permanganate, are placed in a cylindrical dish of about 10 cc. capacity with a 1.5% aqueous solution of silver nitrate; heat the solution until the sections become a yellowish color (about 30 minutes at 60°C.); stir frequently.

10. Wash rapidly in distilled water (5 to 15 seconds).

11. Immerse the sections in another cylindrical dish in Río-Hortega's silver carbonate:

10% aqueous solution of silver nitrate 10 cc.

5% aqueous solution of anhydrous sodium carbonate 40 cc.

The precipitate formed when both solutions are mixed is dissolved with ammonium hydroxide; this must be added drop by drop very carefully; decant when a few particles of precipitate still remain (the excess of ammonium hydroxide must be avoided). Add distilled water to make 150 cc.

Before immersing the sections in Río-Hortega's silver carbonate, add 3 drops of pyridine (if the dish is of 10 cc. capacity). Cover the dish with a watch-glass and heat slowly until the sections become of a blended tobacco-like color (about 20 minutes at 60°C.); stir frequently.

12. Wash rapidly in distilled water (5 to 15 seconds).

13. Reduce in 10% aqueous solution of formalin; the sections are rapidly obscured (black tobacco-like color, which appears in 15 seconds).

14. Wash in distilled water (1 to 15 seconds).

15. Immerse in a 0.2% aqueous solution of gold chloride at room temperature; stirring frequently until the black tobacco-like color changes into gray tones (15 minutes); then heat 10 minutes at 40°C.

16. Fix in a 5% aqueous solution of sodium thiosulfate (1 minute).

Wash in distilled water; dehydrate in 96% alcohol; clear in beechwood creosote; mount in Canada balsam.

Results: The cells and the connective stroma are simultaneously stained. The longer the time in the permanganate solution, the paler the color of the cells and the clearer the contrast of the connective stroma. The precollagen fibrils are stained in black tones, while the collagen bundles are of purplish color.

Río-Hortega's Method for Macrophages

1. Fix in 10% formalin (maximum, 1 month).

2. Cut sections on the freezing microtome.

3. Wash in distilled water (10 to 20 minutes). When the specimen has been fixed for more than 10 days and less than 1 month, it is convenient to leave the sections for 24 hours in distilled water, and even better in a diluted solution of sodium sulfite or in ammoniacal water.

4. Immerse one section in Río-Hortega's silver carbonate for 5 seconds, stirring continuously in order to obtain a homogenous impregnation; change immediately to a 1% aqueous solution of formalin. The results will be different whether the formalin is stirred or not, and both possibilities should always be tested; the sections will be reduced in less than 1 minute if the solution is stirred, but they will need 5 or more minutes for reducing if the solution remains without movement.

A second section is immersed for twice as long in the silver carbonate, and the reduction is made in the same manner. The other sections are left in the silver carbonate for increasing periods of time, doubling the number of seconds with each, the limit being 15 minutes.

After staining, the complete series of sections must be observed under the microscope; the optimum time for the staining of macrophages, which is variable for each specimen, may be determined without mounting the sections and even using low magnification.

5. Wash in distilled water (indefinite time).
6. Tone in the solution of gold chloride.
7. Fix in the solution of sodium thiosulfate.
8. Wash in distilled water. Other complementary staining may be advantageously used at this moment, depending on the structures that are to be observed simultaneously with the macrophages, such as nuclei, fat, hematogenic pigments, etc. Dehydrate, clear and mount.

Steps 6, 7, and 10 are identical with 15, 16, and 17 of the method for pre-collagen fibers.

Results: The cytoplasm of the macrophages is stained in different intensities of purplish color; all of the other structures are weakly stained or invisible, if complementary stains are not used.

Gallego's Method for Elastic Fibers

1. Fix in 10% formalin (indefinite time).
 2. Cut sections on the freezing microtome.
 3. Place the sections in a Petri dish containing about 40 cc. of water; they should be stained promptly, because the water removes the rest of the formalin, some of which must remain in the sections, in order to obtain perfect staining.
 4. Immerse the sections for 5 minutes in:

Water	10 cc.
Pharmacopoeial solution of ferric chloride ($\text{FeCl}_3 + 6\text{H}_2\text{O}$)	5 drops
Nitric acid	1 drop
 5. Transfer sections, without washing, for 5 minutes, to:

Water	10 cc.
Ziehl-Neelsen's carbolfuchsin	30 drops
Nitric acid	1 drop

 This solution and the former (4) must be prepared immediately before using.
 6. Wash rapidly in water (5 seconds).
 7. The sections are differentiated during 5 minutes in the same solution used in step 4.
 8. Wash in water. The sections may be observed under the microscope at a low magnification; the elastic fibers must be the only structures which appear deeply stained. If the staining is pale, steps 4, 5, 6, and 7 may be repeated once or twice. Dehydrate in alcohol, clear in beechwood creosote, wash out the creosote with xylene, and mount in Canada balsam.
- A beautiful and complete counterstain may be obtained by adding the following complementary steps:
9. Immerse the sections for 2 minutes in:

Water	10 cc.
Ziehl-Neelsen's carbolfuchsin	10 drops
Acetic acid	1 drop
 10. Wash rapidly in water (5 seconds).
 11. Immerse the sections for 5 minutes in:

1% aqueous solution of formalin	10 cc.
Acetic acid	1 drop
 12. Wash in water (5 to 15 minutes).
 13. Stain in Cajal's picro-indigo carmine solution (1 minute):

Aqueous saturated solution of picric acid	90 cc.
Distilled water	10 cc.
Indigo carmine	0.25 gm.
 14. Wash in water (15 seconds).
- Dehydrate and mount as before (8).

Results: Elastic fibers, the nuclei, and all basophilic structures appear stained in different tones of violet; the collagen bundles and the acidophilic structures appear blue; the red blood cells and the muscle fibers are a yellowish green.

HISTOGENESIS AND EVOLUTION OF THE TYPICAL SILICOTIC NODULE

According to my observation, the initiation of the nodules takes place around vessels which are reached by the minute sharp silica crystals carried by the alveolar macrophages. At first the process is marked by a proliferation of the reticular (precollagenous, argyrophilic) fibers which appear to form a moderately dense net sustained by a number of thick trabeculae of radiating disposition, which in turn are anchored in a few circular collagenous bands surrounding the central vessel (Fig. 1). This lesion is usually well limited, but the proliferation may extend into the neighboring interalveolar septa. There are always great numbers of desquamated cells of macrophagic character in the adjacent alveoli, and small quantities of albuminous exudate may be present.

While the reticular fibers continue to proliferate rapidly, the central vessel also becomes involved, at first showing signs of obliterating angiitis and finally becoming completely obstructed. The argyrophilic fibers lose their reticular arrangement, become wavy, and are partially transformed into collagenous substance. Through this process there arises a spheroid nodule which stands out clearly from the neighboring alveoli by its hardness and lack of vascularity (Fig. 2).

The greater the number of newly formed collagenous (fascicular, acidophilic) fibers, the slower the further growth of the nodule. When retraction of these collagenous fibers ensues, it causes compression atelectasis of the parenchyma with collapse of the surrounding vessels and bronchi, and there appears a desquamation of alveolar cells of variable intensity. The static period is reached when all of the reticular fibers of the nodule have been replaced by collagenous fibers. Figure 3 shows a large-sized nodule which is still growing (as demonstrated by the persistence of reticular structure), and in it one may observe the intensity of the desquamative process and the widening of the lymphatic spaces. Figure 4 shows a node in which the growth has been arrested; it is made up exclusively of thick collagenous fibers. Here the limiting desquamative process has become organized and static, and the lymphatic spaces are no longer conspicuous.

The first precollagenous fibers to appear in the lesions grow out from the adventitial layer of the small vessels, especially from the venules which have been attacked by silica crystals accumulating in their lymphatic spaces; the subsequent growth of these fibers then becomes autonomous. Other new fibers are formed by the regional histocytes

situated, in order of importance, within the adventitia of small vessels, in the local capillaries, and in the alveolar wall itself.

FACTORS CAUSING THE POLYMORPHISM OF THE SILICOTIC NODE

In only a few cases is the structure of the silicotic nodule as simple as in the foregoing description since it is subject to modification by many factors.

Several incipient nodules may be so close to one another that their proliferating reticular fibers coalesce and they become fused into the likeness of acini or clover leaves (Fig. 5). This image is probably the result of the simultaneous proliferation around several vessels joining two main nodules, each one of them composed of smaller secondary ones. New nodules sometimes appear near the border of other more advanced lesions. In Figure 6 there are two such nodules incompletely separated from an older one by a few collapsed alveoli. This mechanism of spreading of the silicotic nodule may be termed *growth by apposition*.

There is not always as sharp a limit between the older nodule and its satellites as in the foregoing example. Figure 7 shows the edge of a silicotic nodule still in the precollagenous stage which grows outward irregularly through the formation of secondary nodules, with multiplication of fibers alongside the vessels and invasion of the alveoli. Such formations may indicate a continuous arrival of siliceous dust in the alveoli and its accumulation around the vessels irrigating the border of a nodule.

Another factor contributing to the progression of the lesions is the limiting desquamative pneumonia which is a constant companion of spreading silicotic nodes. Figure 8 illustrates such a process by showing the marginal alveoli of a nodule filled with desquamated cells and scanty albuminous exudate of vascular origin. The relative integrity of the interalveolar septa is useful in differentiating this aseptic irritative process from lesions caused by either banal or tuberculous infection.

With adequate technics and high magnifications the desquamated cells are shown to have pseudopodial forms, which indicate active migration and, therefore, macrophagic character (Fig. 9). The exudate itself is often free of blood corpuscles. The small, round, often pyknotic nuclei, and the products of cytorrhesis which appear in the photomicrograph belong to desquamated cells and only exceptionally to leukocytes or lymphocytes.

Desquamative pneumonia contributes to the spread of silicotic lesions because its exudate tends to become organized. Figure 10 shows the delicate reticular fibers largely originating in the wall of the alveoli and partly formed by the desquamated histocytes. As they replace the

intra-alveolar fluid there appears a reticulum similar to the structure of the neighboring nodes; the new reticulum slowly coalesces with the older nodes as shown in Figure 11. In some regions where the alveolar wall is still preserved the new reticulum is quite definite, but in other sites the boundary between the silicotic nodes and the organized exudate has disappeared. This second type of growth may be defined as *growth by organizing pneumonitis*.

The third and last factor causing polymorphism of the lesions is the presence of atelectatic areas around the nodes. If compression were the only cause of atelectasia, one should not expect the latter to contribute to the growth of the lesions, but there are also inflammatory phenomena due chiefly to the presence of siliceous dust in the alveoli and to the ischemia resulting from the compression of the blood vessels. These inflammatory phenomena also contribute to the early destruction of the alveolar epithelium and thus break down the barrier between the reticular fibers of the interalveolar septa and the lumina of the alveoli which contain the irritative factor. This breakdown starts proliferation of the fibers. Finally, fibrils from opposing walls become fused and a solid mass arises which comes into contact with neighboring nodes as shown in Figure 12. This *growth by transformation of atelectatic zones* can be distinguished from both of the previous types of growth by one significant detail: whereas in growth by apposition and in growth by organizing pneumonitis the structure of the node is predominantly reticular, collagen transformation must have taken place in order to produce adequate zones of atelectasia.

OBSERVATIONS ON THE STRUCTURE OF THE SILICOTIC NODE

Silver impregnation technics have revealed details referring mainly to the intranodular blood vessels, the connective fibrils, the process of central softening, and to the activity of the macrophages.

The most frequent change in the *intranodular blood vessels* is the widening of the adventitial lymphatic space. In Figure 13 the central vessel of the node has been cut longitudinally and the endothelial and muscular layers show little change, while the perivascular lymphatic space appears as a wide channel conspicuous by its net of reticular fibers. Figure 14 shows a similar vessel cut transversely. Within the dilated space there may be small numbers of lymphocytes, macrophages, and silica crystals. I believe that these elements may be carried there passively following the physiologic draining current from the alveoli.

Alterations of the vascular intima begin somewhat later. In Figure 14 there is already a slight proliferation of the endothelium with conse-

quent narrowing of the lumen. Figure 15 illustrates the period of maximal proliferation and shows the lumen completely obliterated. This vascular obliteration may be responsible for the transformation of precollagen fibrils into collagen, to be discussed later in this paper.

The degenerative changes of intranodular vessels are often compensated by a new growth of capillaries originating from vessels situated outside of the lesion. The new capillaries may have to traverse wide distances and they behave similarly to, but not exactly like, capillaries arising in canalizing thrombi or in foreign body reactions. They are difficult to demonstrate with aniline stains but appear clearly when silver impregnations are used, as in Figure 16 where they appear as delicate tubes of precollagen fibrils. Since this section is very thin, only a minority of these newly formed capillaries can be discerned. Their real number is more apparent when retraction takes place and the density of the structures increases, as in Figure 17 in which the nodule is invaded by a new net of capillaries penetrating it from outside. The relation between this process and the softening of the silicotic nodule will be discussed later.

The *structure of the reticular network* of the silicotic node is quite variable. When the transformation into collagen has progressed but little, the argyrophilic fibers preserve their characteristic reticular aspect (Fig. 18); the radiating disposition previously described is often very clear. Sometimes at the border of the growing nodule, sometimes in its center and near the radiating zones, the fibers become more independent and show a tendency to form wavy bundles; this indicates the first change in their chemical composition, also shown by their avidity for acid dyes. Soon after, they break up into parallel bundles and acquire the histologic characteristics of collagen fibers (Fig. 19). The number of straight reticular bundles increases and it is common to find nodules like that in Figure 20, which consist of fibers of trabecular disposition but which otherwise conserve their precollagen characteristics. One must be careful to distinguish these bundles of wavy precollagen fibers, which mark the beginning of collagenous transformation, from those entirely straight fibers which are the result of mechanical traction (Fig. 21). The preponderantly parallel arrangement here is due to the displacement resulting from the retraction of the older collagen bundles in the neighboring nodules.

In Figure 22 are found the first collagen bundles arising from the wavy fibrils in the center of a silicotic nodule. Here also, as occurs usually when collagen is formed by an abnormal mechanism, the fibers soon become hyalinized and contracted. This causes the whiteness and hardness of the nodules that characterize their macroscopic appearance.

However, there are also autolytic phenomena which tend to produce the opposite result. The softening of completely fibrous nodes is due in part to the formation of new capillaries and in part to the proteolytic action of the phagocytes contained in the lesions. Figure 23 is taken from such a softening nodule; in the loose zone the central vessel and débris of dissolving reticular fibers may be observed. In Figure 24 the central area is filled with lymphoid cells and the penetration point of the vessels which have produced the softening can be distinguished in the lower part of the figure. In Figure 25 there are numerous active macrophages beside the vessels and lymphocytes. The last three illustrations taken together show the main features of softening in successive stages.

Among the many elements found in softening foci, cellular débris, masses of siliceous crystals, and fragmented fibrils can be observed. There are also some elements whose significance and mode of origin are not clear, as, for instance, the chrysophilic granules shown in Figure 26; they have irregular shapes with generally rounded contours and they stain deeply with warm gold chloride. Their appearance would have suggested derivation from erythrocytes but for the lack of any other sign of hemorrhage in the softened silicotic nodules. Another problematic feature concerns the large refringent crystals with indefinite edges (Fig. 27) which are occasionally present. They show varying degrees of affinity for the basic aniline dyes, as do calcium salts, and they are found in the vicinity of other basophilic granules. They resist treatment with both alcohol and xylene.

Study of the *macrophages* with the specific silver impregnation techniques of Río-Hortega has contributed much valuable data about the histogenesis and evolution of the silicotic nodule. Macrophages appear quite early surrounding the vessels of a developing nodule (Fig. 28). Many of them come from the alveoli and transport the siliceous crystals into the adventitial spaces where the silicotic lesions begin, but not all of the crystals arrive in the adventitial space in this manner and probably a considerable number of the perivascular macrophages are formed *in situ* by the histocytes surrounding the altered venules. When the irritation by the dust particles excites the reticular fibers to proliferate, the adventitial space soon becomes obliterated; thus, the irritating dust particles are accumulated around the vessels where they stimulate the formation of more macrophages. The number of macrophages increases progressively until there are large masses of ameboid cells containing cellular detritus (Fig. 29).

The detailed structure of the macrophages can be observed in Figure 30, taken at a higher magnification. The cytoplasm is highly developed

and shows rounded shapes. The nuclei are hidden by the numerous intracellular inclusions, including siliceous crystals. Sometimes there are small vacuoles that give the microchemical reactions of the fatty substances and some of the pseudopodial prolongations are found detached from the main body of the cells.

When the reticular fibers are transformed into collagen there begins a rapid destruction of the macrophages. Some of them are caught between the meshes of the hyalinizing bundles during their process of hardening and retraction and space becomes too limited even for cells as stereotropic as these. Figure 31 shows the macrophages imprisoned between the thick hyalinized collagen bundles. However, the greater part of the destruction takes place in the softened areas by autolytic changes (Fig. 32); the intensity and speed of the regressive phenomena can be deduced from the pyknotic nuclei and liquefying cell bodies.

The previously well known fact of the disappearance of the elastic fibers has been further confirmed by my observations. Figure 33 from a preparation stained by Gallego's method shows the absence of elastic fibers in the nodular zone and in addition their radiating orientation towards the periphery of the nodule as a result of the traction exercised by the shrinkage of the newly formed collagen fibers.

DIFFUSE SILICOTIC LESIONS

Fibrous nodules are the most characteristic type of lesion in silicosis, but other types are present. Nodules are frequently found together with diffuse fibrous plaques, peribronchial sclerosis, and with focal pleural thickening.

Diffuse fibrous plaques may be interpreted as nodules profoundly modified by vascular proliferation, migration of macrophages, and superimposed inflammatory lesions (Fig. 34). Among the inflammations, tuberculosis apparently plays the most active part in the transformation of lesions into extreme fibrous plaques. The collagenous network of these plaques is so irregular that retraction has few consequences and there is only slight deformation of alveoli, vessels, and bronchi.

Peribronchial sclerosis is probably a product of the same factors which excite the formation of the nodules, namely, the penetration of particles of silica into the lymphatic spaces; but, since the efficiency of lymphatic drainage is lower in the vicinity of the bronchi and there is reticular connective tissue within their mucosa, well defined nodules are the exception rather than the rule (Fig. 35). There is an intense desquamation of the mucosa and the lumen of the bronchi contains exudate, although it must be noted that these phenomena are exaggerated by post-mortem autolysis due to delay in fixing the specimen.

The *pleuritis* of silicosis often appears rather early, sometimes being the first anatomic change of the disease. It begins by a moderate thickening of the visceral pleura with considerable dilation of its lymphatics and blood vessels. The hypertrophy of the connective structure is relatively slow and usually the reticular network, which characterizes the parenchymal lesions of silicosis, fails to develop. The neighboring alveoli are not affected until late in the process (Fig. 36).

When pleural thickening has reached full development, as in Figure 37, the surface of the serous membrane is found covered with newly formed capillaries together with a delicate network of reticular fibers. These fibers are formed by wandering histocytes, many of which show the changes of fibrogenesis. The vessels, especially the lymphatics, are always dilated. The lesions show a tendency to limit themselves to plaques which can be observed macroscopically; there is either very little hyalinizing of the collagen fibers or none at all. The presence of silicotic nodules in the immediate vicinity of the pleural plaques seems to increase the size of the latter. It is to be assumed that the pleural lesions have the same significance as the perivascular nodules; they are also a reaction to the presence of siliceous crystals, either free or carried by macrophages, arriving through the lymphatic stream.

SUMMARY

After studying the structure and evolution of the silicotic node with the silver impregnation methods of Río-Hortega, the following conclusions have been reached.

1. The histologic basis of all lesions induced in the human lung by siliceous dust is the proliferation of the reticular fibers. After proliferation these fibers are partially or wholly transformed into collagen and undergo hyalinization and retraction.

2. The silicotic node continues its growth as long as there are reticular fibers left; growth is arrested when collagenization is complete.

3. The reticular fibers of the nodule originate mainly in the adventitial layer of small vessels, especially the venules which have been attacked by siliceous dust deposited in their adventitial lymphatic spaces. Fibrogenesis from regional histocytes is of secondary importance.

4. The nodules grow by (a) apposition of adjacent nodules in the later stages of development, (b) organization of the exudate of the desquamative pneumonia which marks the limit of the developing nodules, and (c) transformation of adjoining atelectatic zones.

5. The adventitial lymphatic space of the blood vessels is frequently found dilated and obstructed by proliferating reticular fibers. Sometimes the nodes are penetrated by newly formed capillaries arising from nearby interalveolar septa.

6. Many of the silicotic nodules undergo a softening process due to the penetration of newly formed capillaries and to the autolytic action of macrophages. In the softening foci I have found chrysophilic masses and basophilic crystals whose origin and significance cannot be interpreted at this moment.

7. The macrophages found in silicotic lesions are first seen around the vessels, shortly after the beginning of the proliferation of reticular fibers. They contain great amounts of detritus and siliceous particles. They are destroyed later when they are compressed by bundles of hyalinizing collagen and also in great part by autolysis.

8. The elastic fibers disappear in the completely constituted lesions. Their disposition outside the latter is indicative of the traction originated by the shrinking of silicotic nodules in the stage of collagenous transformation.

9. Beside the typical silicotic nodule there also appear diffuse sclerotic plaques, peribronchial sclerosis, and proliferative pleuritis. These three lesions have special characteristics which can be related to the different types of lymphatic drainage pertaining to each region; however, their histogenesis, structure, and evolution are similar to those of the typical silicotic nodule.

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DESCRIPTION OF PLATES

PLATE 5

- FIG. 1. Silicotie nodule in its initial stage, showing proliferation of the reticular fibers around a vessel. The lumina of the alveoli contain great numbers of desquamated cells and some albuminous exudate. $\times 110$.
- FIG. 2. Silicotie nodule in evolution. The central vessel is obliterated. The connective fibers are no longer arranged in reticular form but are in bundles and are partially transformed into collagen. $\times 110$.
- FIG. 3. Silicotie nodule of greater size than that shown in Figure 2 but still consisting mainly of reticular fibers. The desquamative pneumonitis in its periphery and the dilatation of lymphatic spaces indicate the evolutive character of the lesion. $\times 70$.

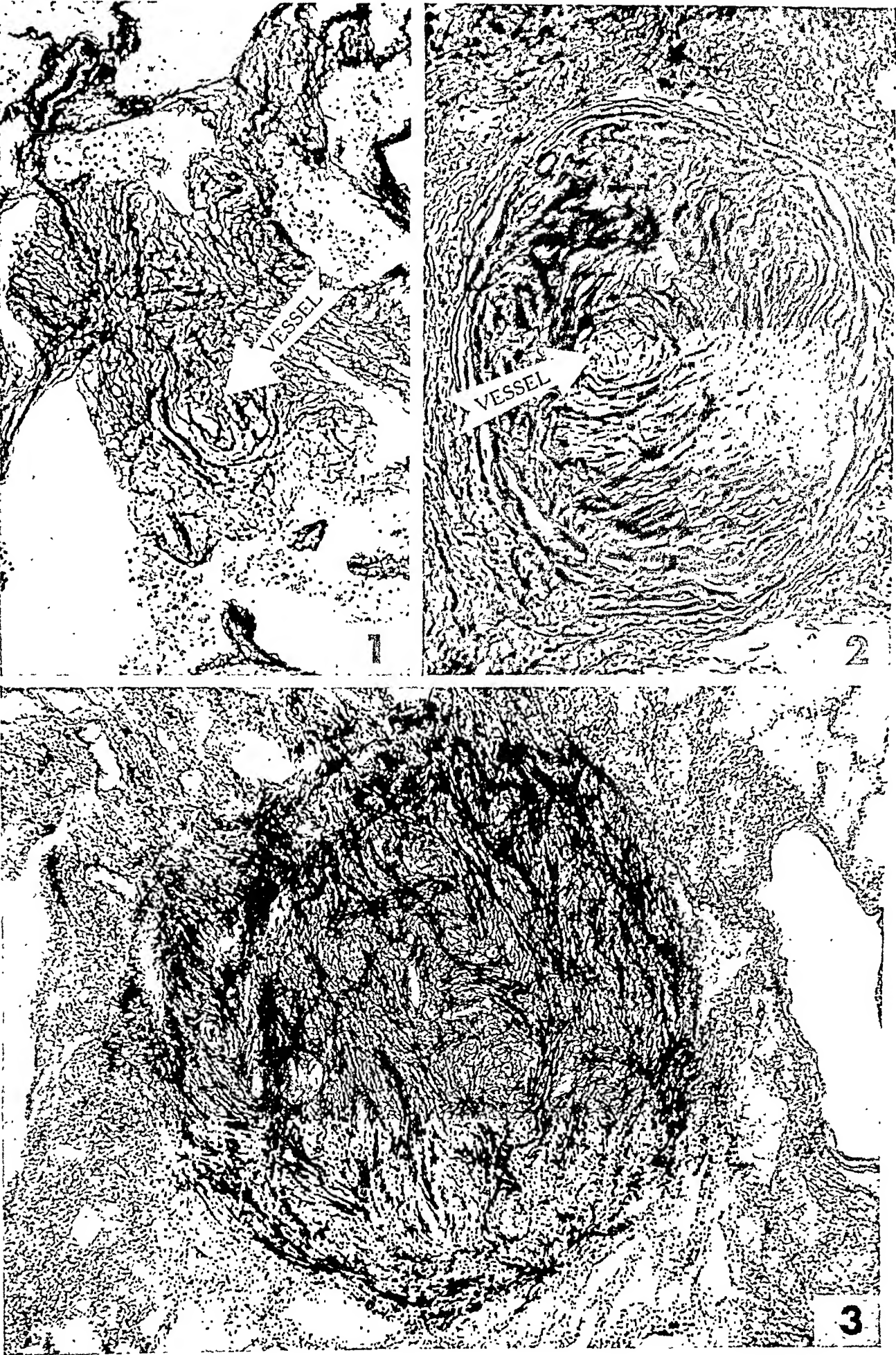


PLATE 6

- FIG. 4. Stationary silicotic nodule composed exclusively of thick collagen bundles. The limiting pneumonitis has become organized and the lymphatic spaces are not patent. $\times 70$.
- FIG. 5. Acinous silicotic nodule produced by the coalescence of two primary nodes, each one of them integrated by smaller secondary ones. It is difficult to define their limits because of the promiscuous interlacing of the reticular fibers. $\times 110$.
- FIG. 6. Silicotic nodule growing by "apposition." Incipient nodules appear near the edges of a more advanced lesion. The limit between the new lesion and the old is marked by a few collapsed alveoli. $\times 110$.

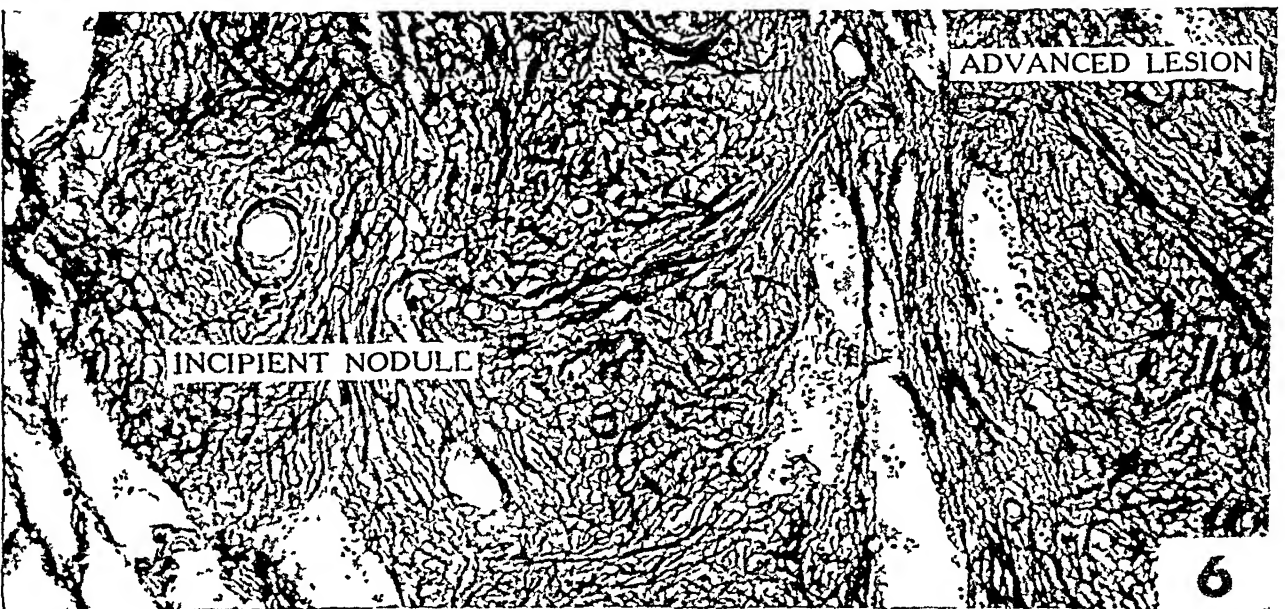
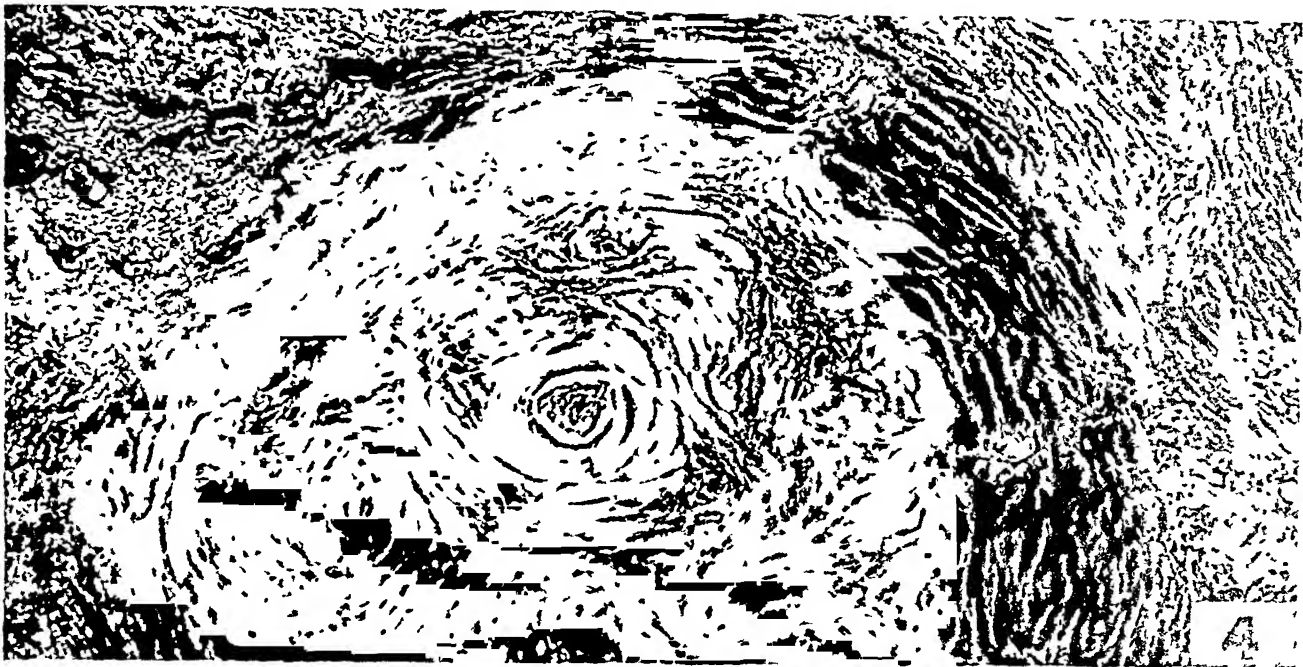


PLATE 7

- FIG. 7. Edge of an advanced silicotic nodule growing by "apposition." The limit between the new lesion and the older one is less distinct than in Figure 6. \times 110.
- FIG. 8. Silicotic nodule growing by organization of desquamative pneumonitis. The alveoli contain desquamated cells and a small amount of albuminous fluid. \times 110.
- FIG. 9. Detailed view of the desquamative pneumonitis around a silicotic nodule in evolution. Alveolar macrophages are seen, some of them in necrobiosis, the majority in activity and with pseudopodial prolongations. \times 440.

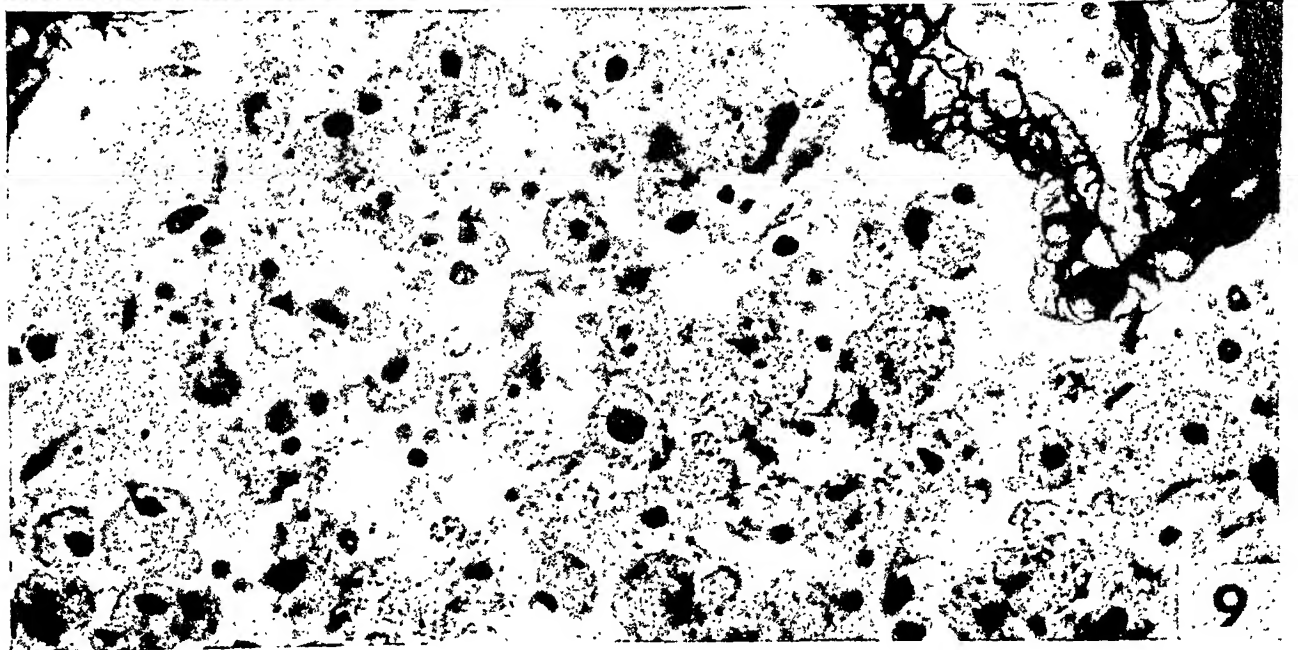
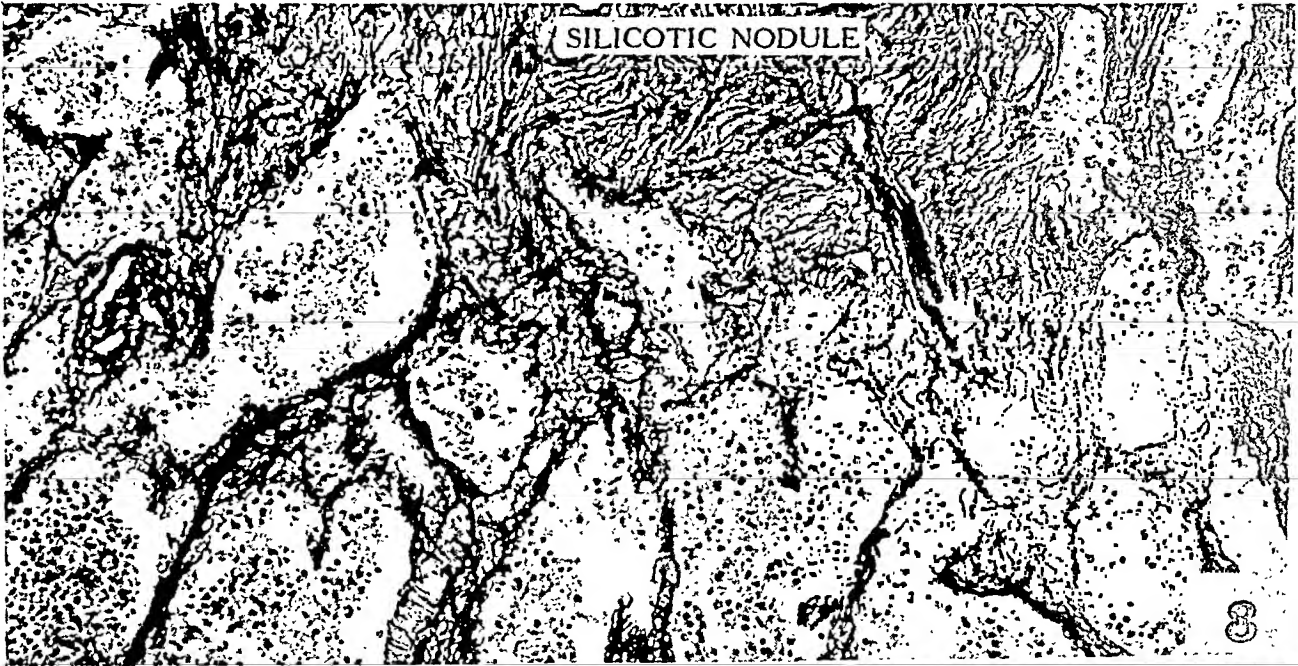
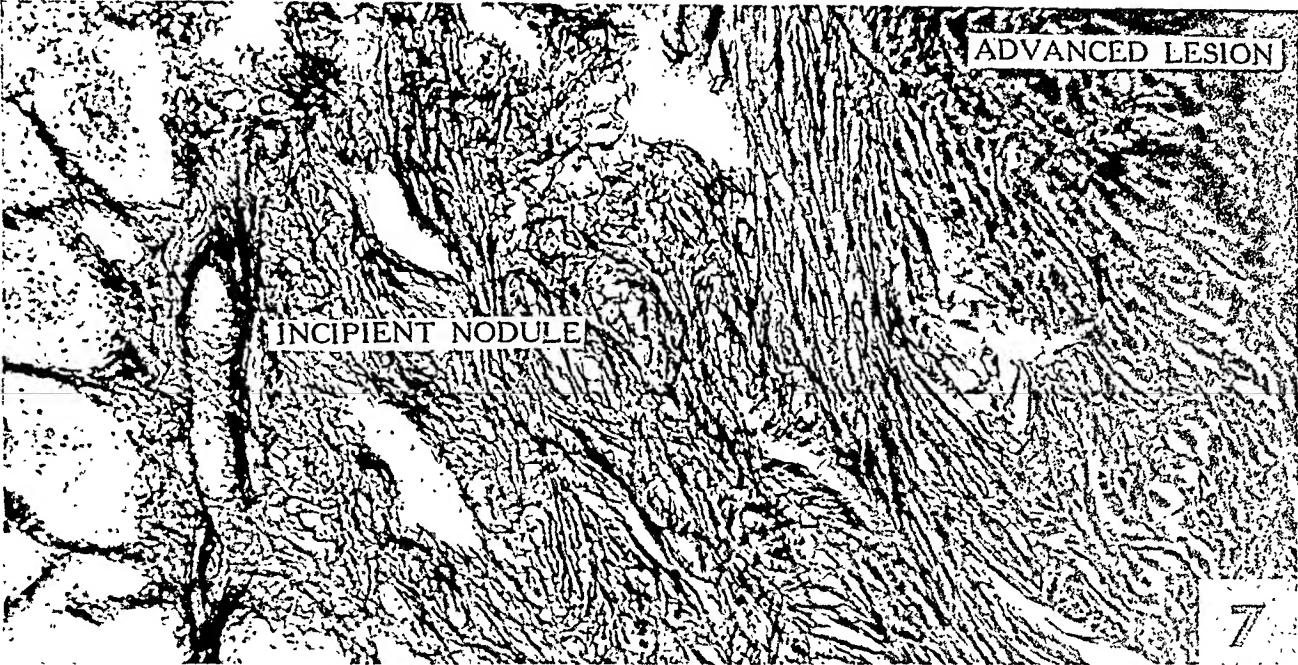


PLATE 8

- FIG. 10. Organization of desquamative pneumonitis. The intra-alveolar fluid is being replaced by delicate reticular fibers, in part growing out from the reticulum of the alveolar walls and in part produced by desquamated histocytes. $\times 220$.
- FIG. 11. Limiting pneumonitis already organized and in coalescence with the edge of an older silicotic nodule. The partial conservation of the alveolar walls makes it possible to distinguish the pneumonic area. $\times 70$.
- FIG. 12. Growth of a silicotic nodule by transformation of atelectatic zones. In the right upper corner there is the border of an old collagen nodule; the remainder of the field is occupied by the atelectatic area. $\times 110$.

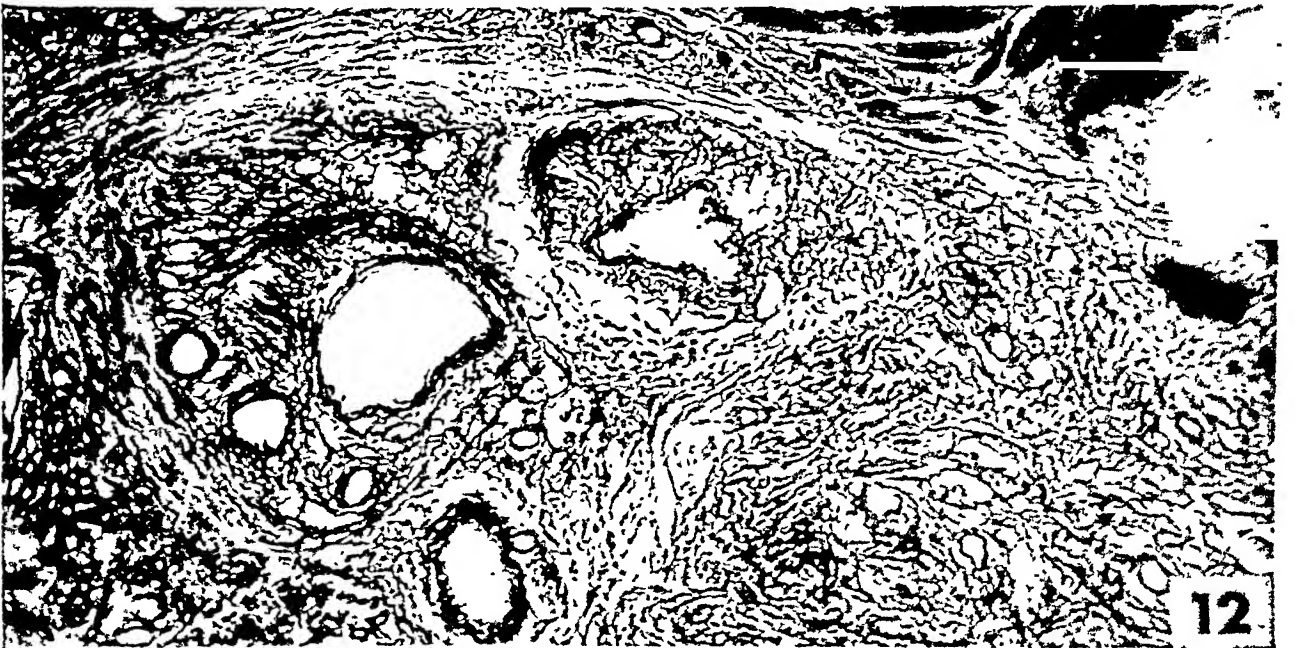
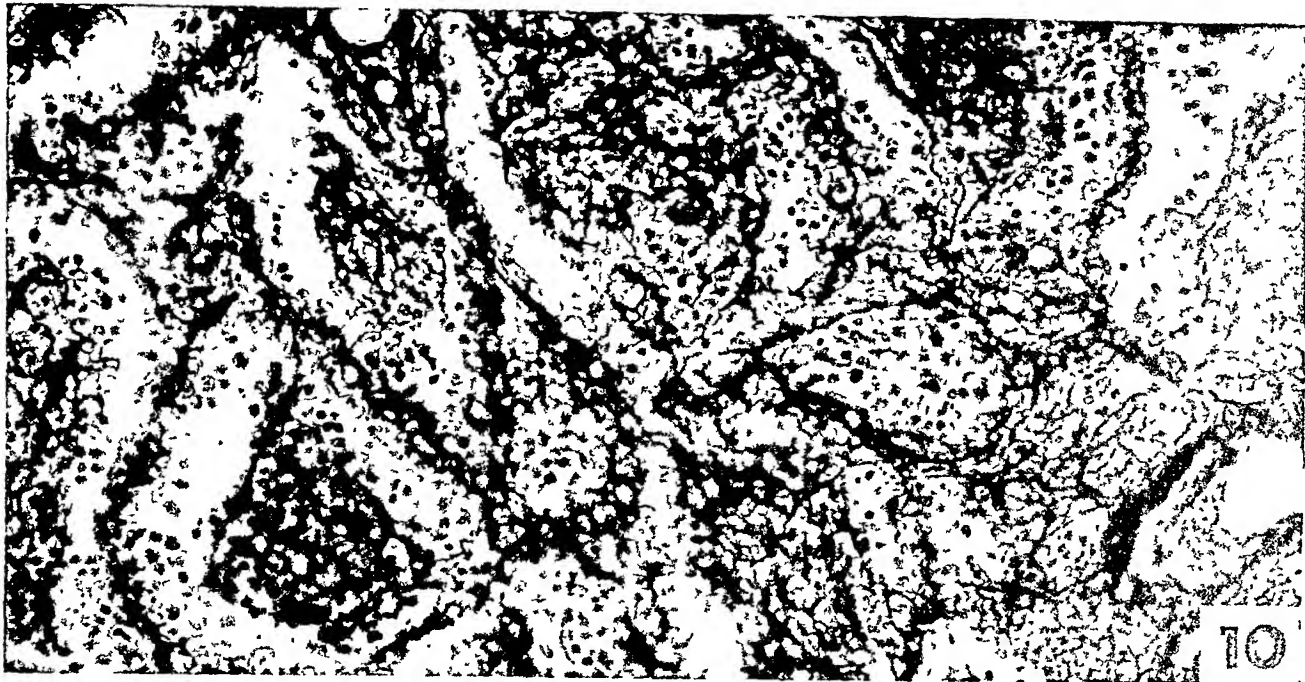


PLATE 9

For "lympathic" read "lymphatic."

FIG. 13. Dilatation of the adventitial space of a blood vessel cut longitudinally within a silicotic nodule. The reticular fibers of the lymphatic space have proliferated. $\times 220$.

FIG. 14. Another vessel similar to the one shown in Figure 13, but cut transversely. In the adventitial lymphatic space, which is dilated and almost obstructed by reticular proliferation, there are a few lymphocytes, macrophages, and siliceous particles. $\times 220$.

FIG. 15. Central vessel of a silicotic node with more advanced changes than those in the preceding figures. The intima has proliferated and the lumen is completely obstructed. $\times 220$.

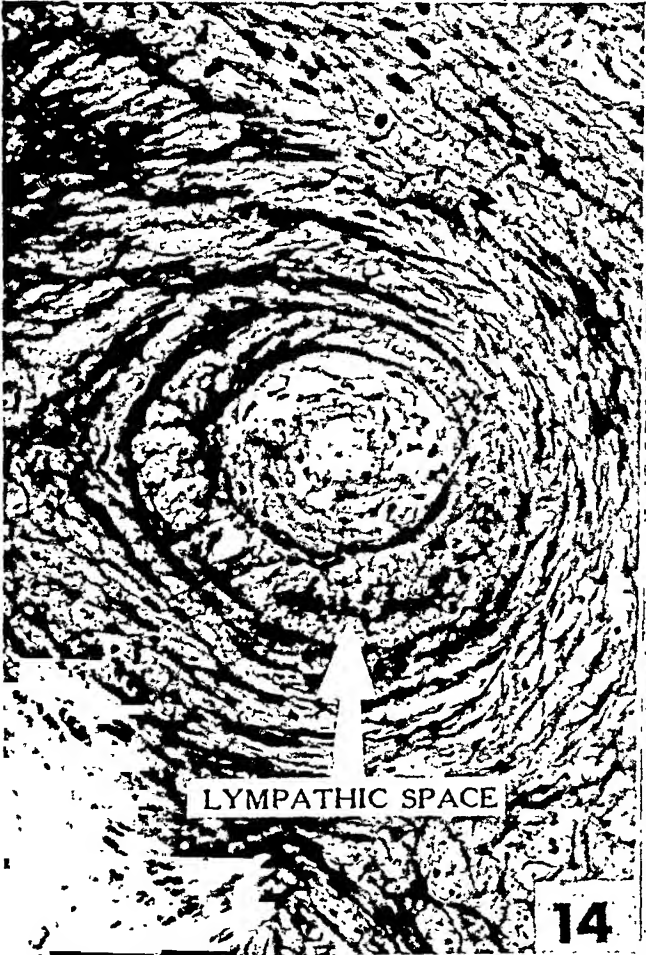
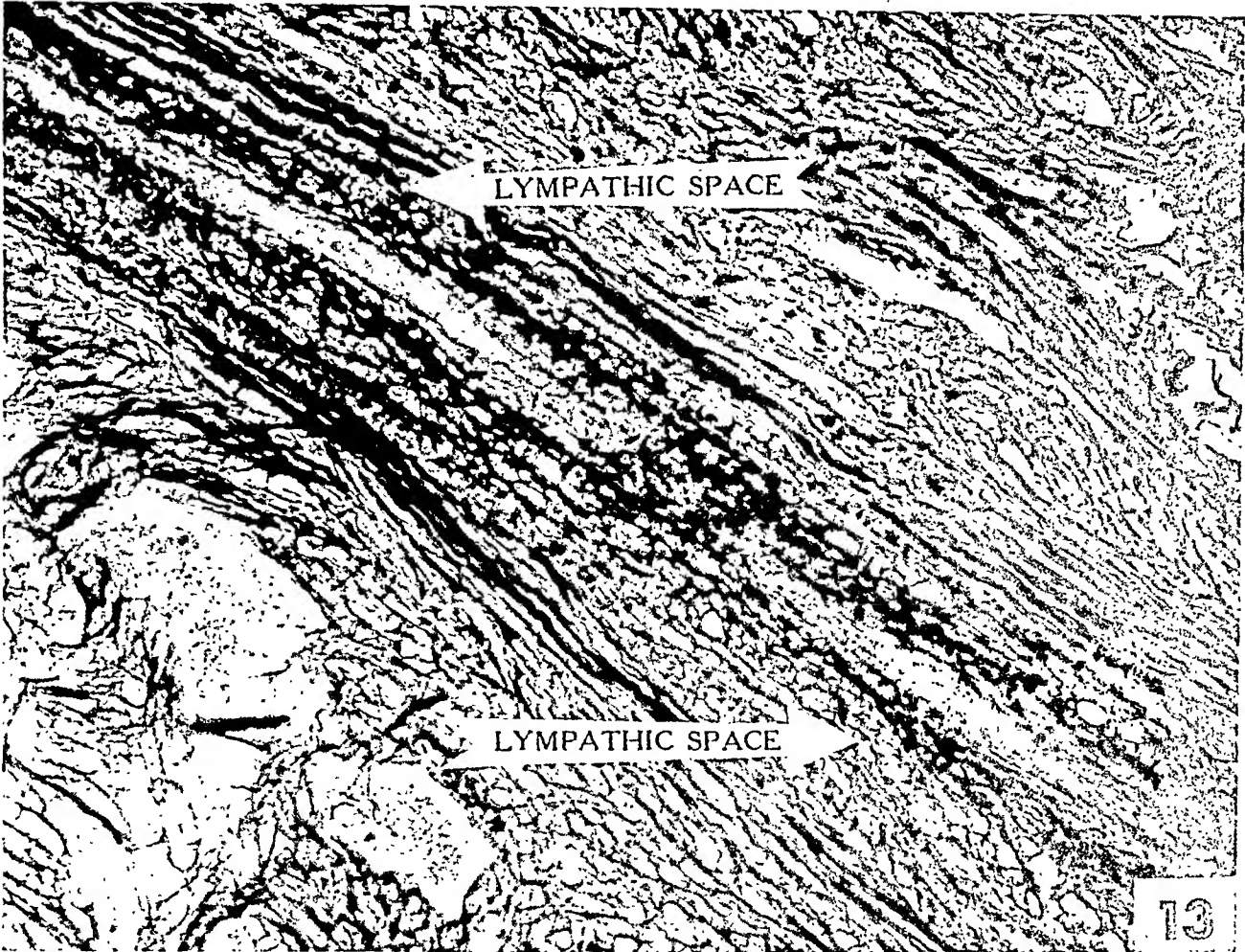


PLATE 10

- FIG. 16. New formation of capillaries in a silicotic node in evolution. The vessels are clearly seen among the meshes of the reticular fibers and are similar to those observed in organizing thrombi and in foreign body reactions. $\times 110$.
- FIG. 17. Silicotic nodule during the process of collagen transformation with great numbers of newly formed capillaries arranged in a dense net of multiple anastomoses. Vascular proliferation contributes to the conservation of the reticular fibers. $\times 110$.
- FIG. 18. Persistence of the characteristic reticular structure of the argyrophilic fibers within a large silicotic nodule. Only a few filaments show a fascicular disposition. $\times 220$.
- FIG. 19. In this silicotic nodule the reticular structure of the argyrophilic fibers is partially lost. The fibers form parallel bundles without anastomoses, as is characteristic for collagen fibers. $\times 220$.

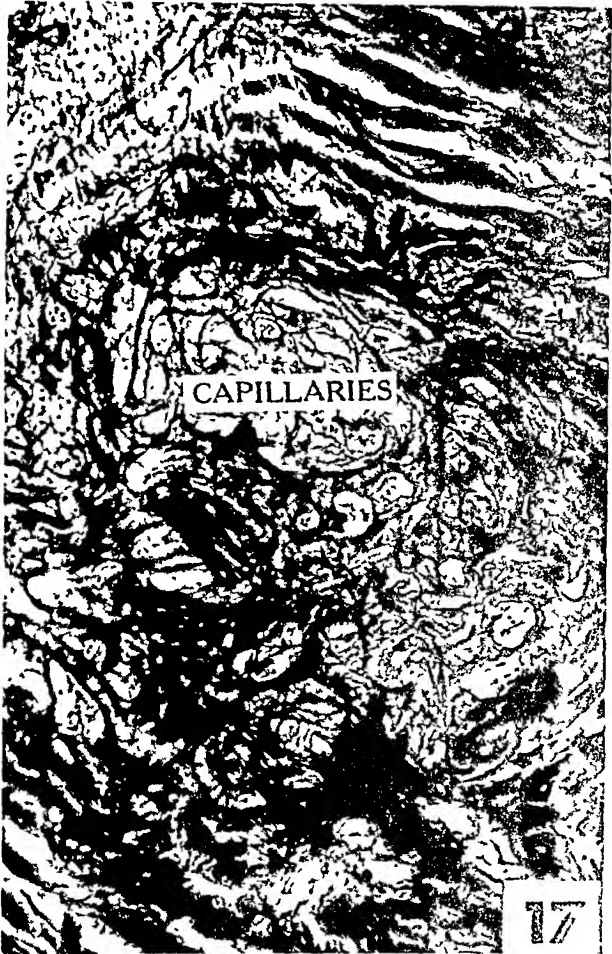
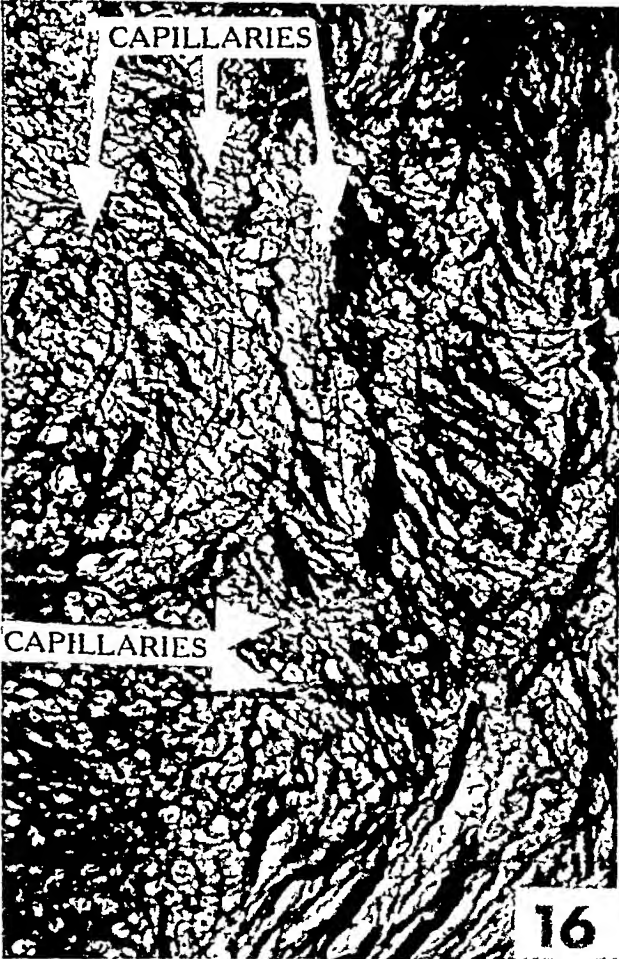


PLATE II

- FIG. 20. Connective structure of a silicotic nodule at the beginning of collagenous transformation. There are several bundles of fibers but between them the picture is still predominantly reticular. $\times 110$.
- FIG. 21. Straight reticular fibers due to traction as a consequence of the shrinkage of the collagen fibers in neighboring silicotic nodules. $\times 220$.
- FIG. 22. Appearance of the first collagen bundles formed from the reticular fibers in the center of a silicotic nodule. The collagen fibers can be distinguished by their greater thickness, rectilinear arrangement, and lack of anastomosis. $\times 110$.
- FIG. 23. Center of a softening silicotic nodule at high magnification. In the loose area there are the débris of destroyed collagen fibers and a newly formed blood vessel. $\times 220$.

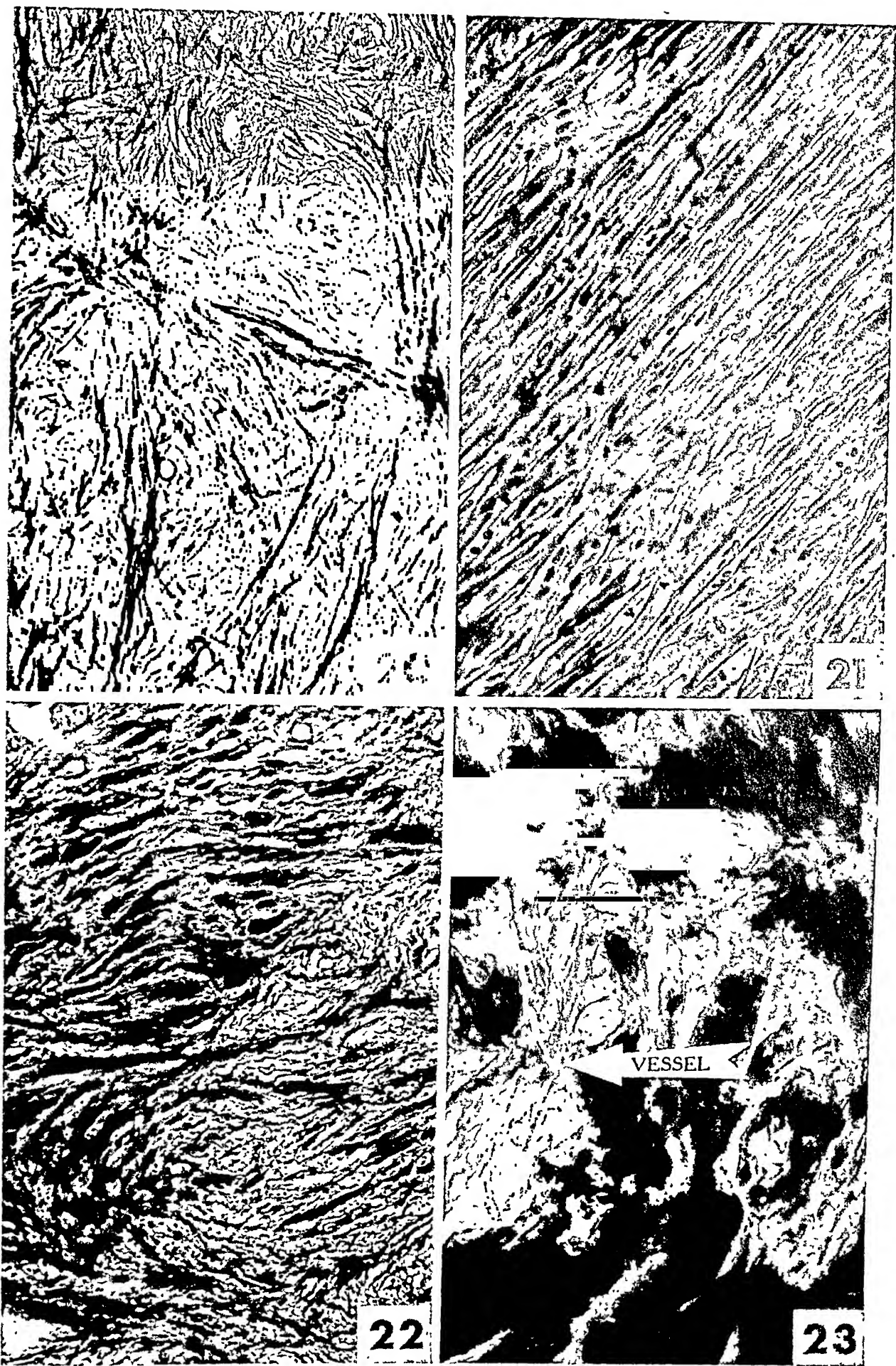


PLATE 12

- FIG. 24. Small silicotic nodule with its softening center occupied by lymphocytes. The canalizing vessel penetrates from the lower part of the field. $\times 110$.
- FIG. 25. To the vessels and lymphocytes in the softened center of the nodule, large quantities of active macrophagic cells have been added and their proteolytic enzymes have begun to destroy the collagen. $\times 110$.
- FIG. 26. Irregular chrysophilic masses with rounded edges, of unknown origin and significance, which are found between the fibers in the softened areas of some silicotic nodules. $\times 440$.
- FIG. 27. Large crystalline masses with indefinite edges, which sometimes appear in the softened areas of advanced silicotic lesions. $\times 680$.

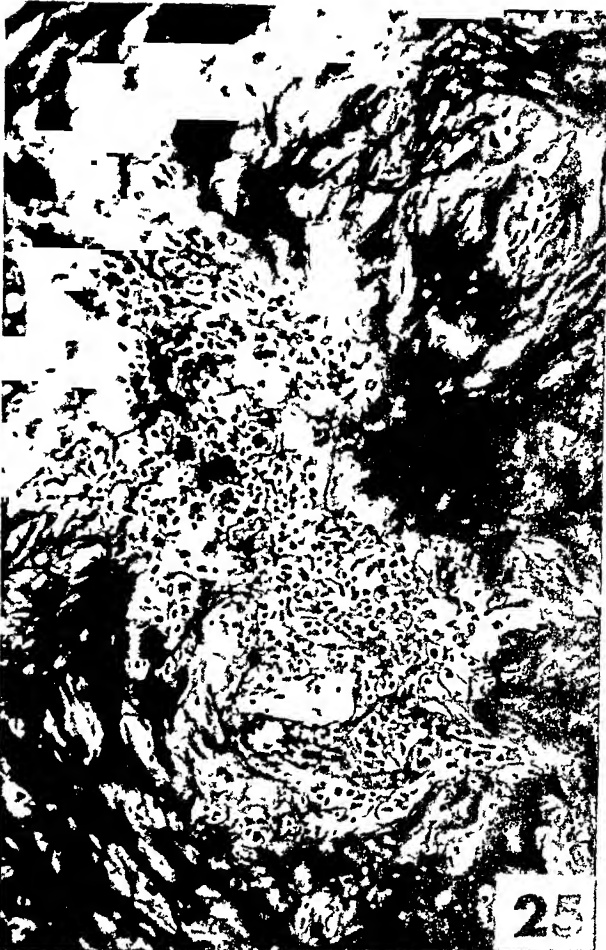


PLATE 13

- FIG. 28. Macrophages arranged around the lymphatic vessels. These macrophages have their origin mainly from the histocytes of the alveolar walls. They are the carriers of the siliceous particles. $\times 110$.
- FIG. 29. Dense accumulation of macrophages as seen at a higher magnification than in Figure 28, showing the cytoplasm filled with siliceous particles. The accumulation of these cells is explained by the obstruction of lymphatic drainage. $\times 220$.
- FIG. 30. Detailed structure of the macrophages contained in silicotic nodules. The cytoplasm is highly developed and has a rounded shape. The phagocytized particles almost hide the nucleus. $\times 440$.
- FIG. 31. Macrophages caught between the hyalinizing collagen bundles; the retraction of these bundles contributes to the compression of the cells placed between them and thus to the atrophy of all living elements of the silicotic nodule. $\times 220$.

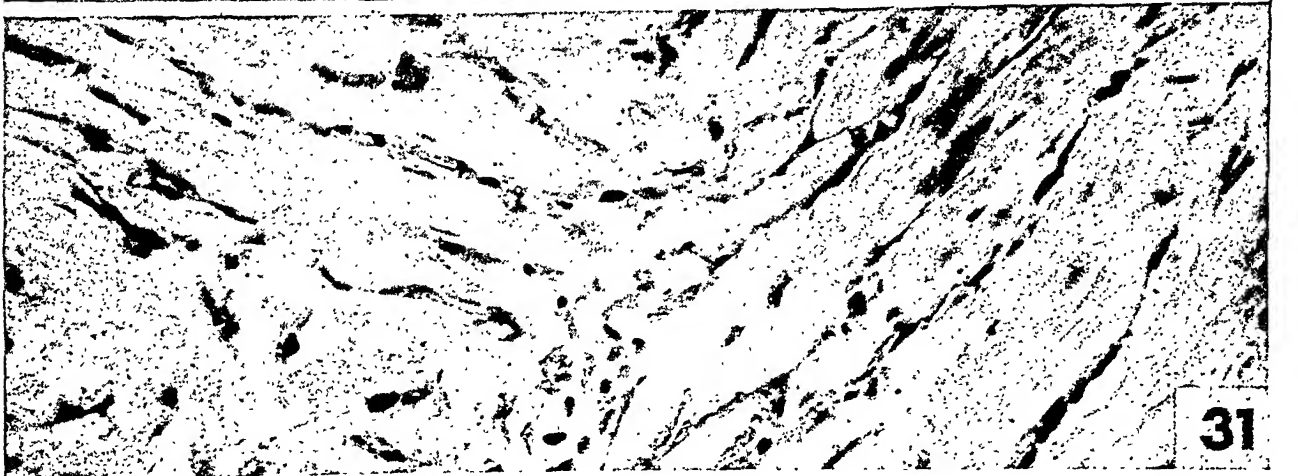
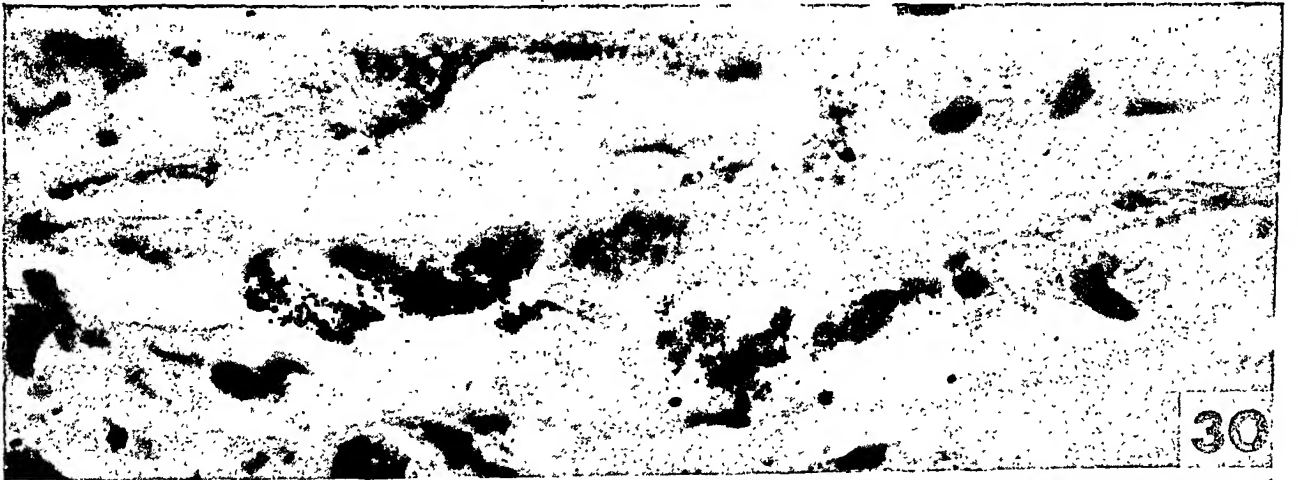
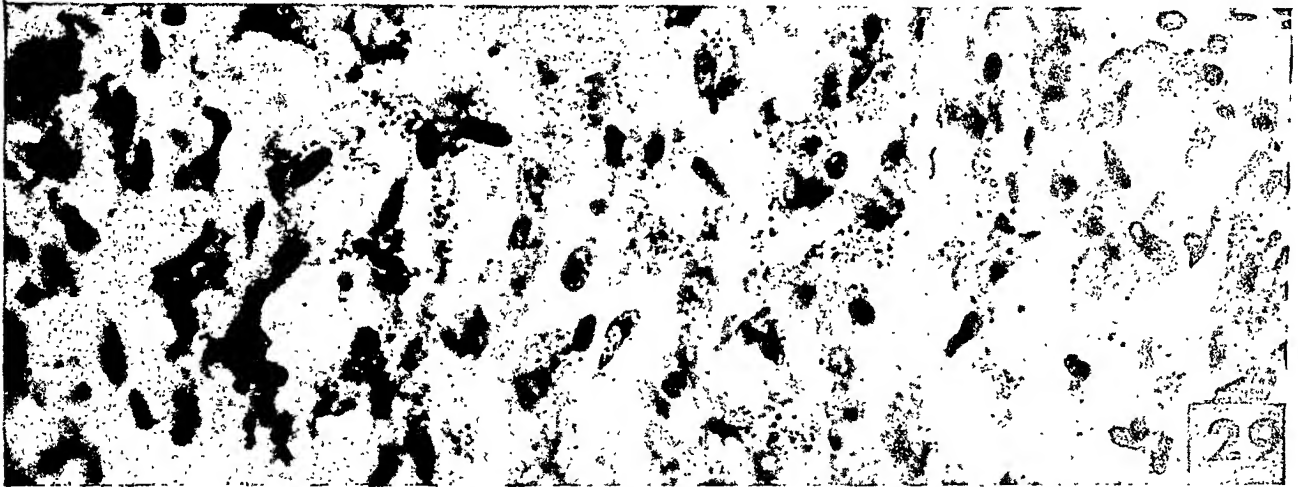
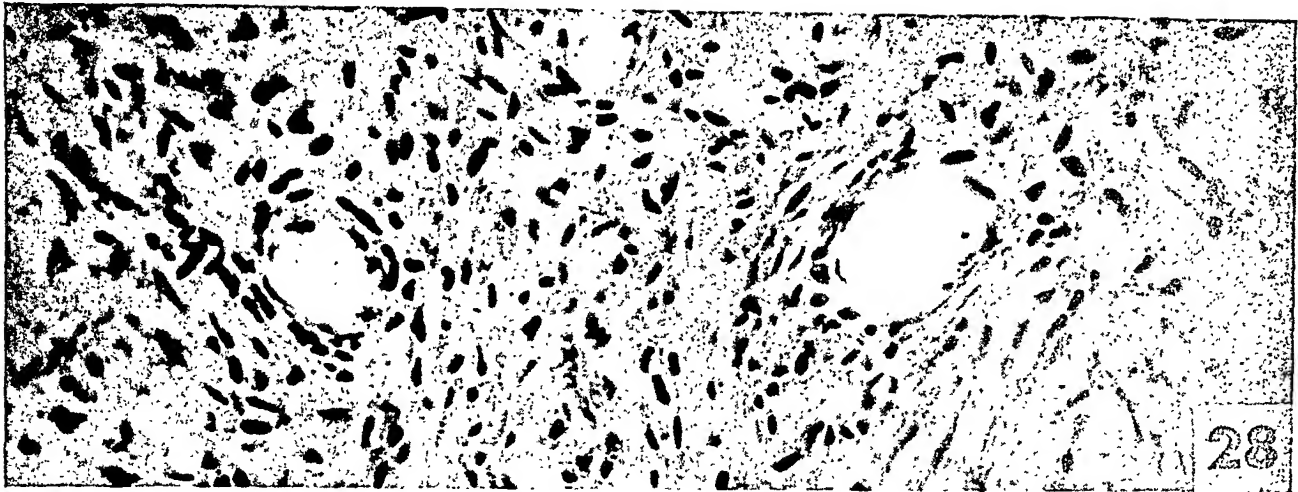


PLATE 12

- FIG. 32. Macrophages in necrobiosis, with pyknotic nuclei and liquefying cytoplasm, within the softened area of an old silicotic nodule. The destruction of the cells in this case is due to autolysis, not to compression. $\times 220$.
- FIG. 33. Pulmonary tissue which contains a silicotic nodule, stained for elastic fibers. The fibrous part is lacking in elastic fibers, and in the neighboring alveolar walls the elastic fibers are distorted by the retraction of the sclerotic lesion. $\times 70$.
- FIG. 34. Diffuse sclerotic plaque representing a silicotic nodule deeply altered by vascular proliferation, multiple softening processes, and inflammatory phenomena. $\times 110$.

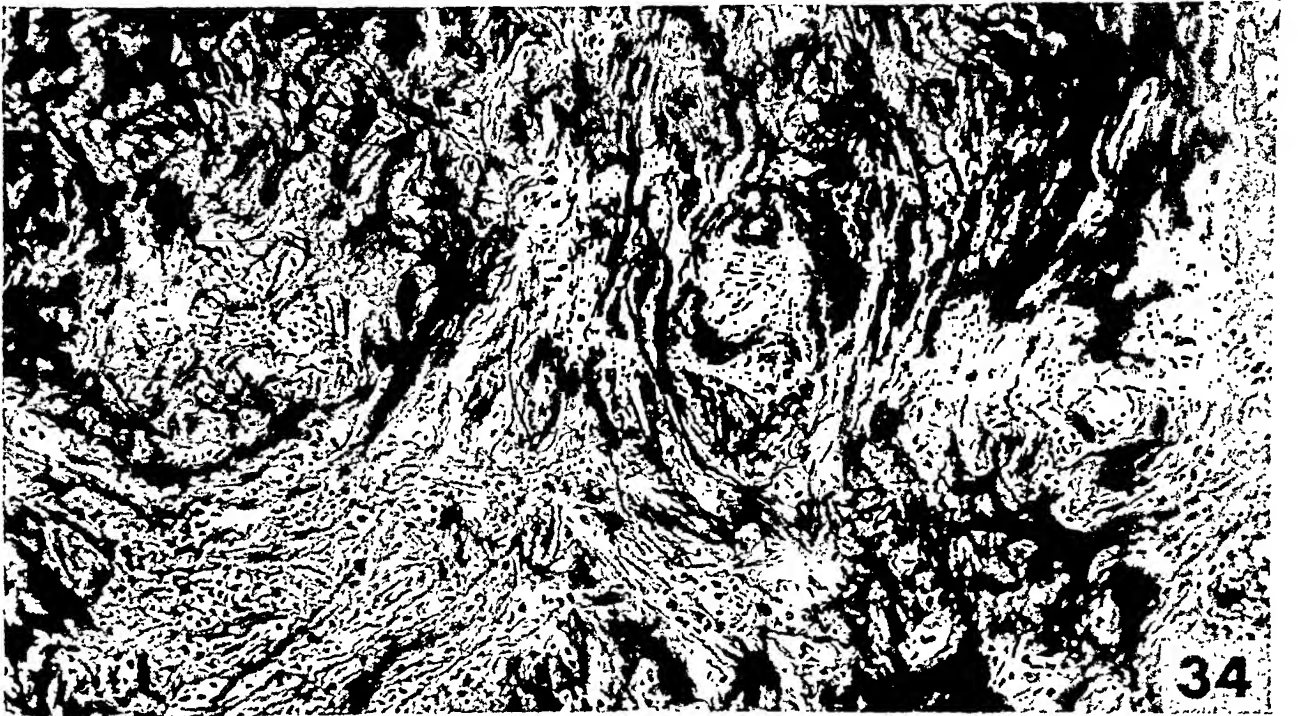
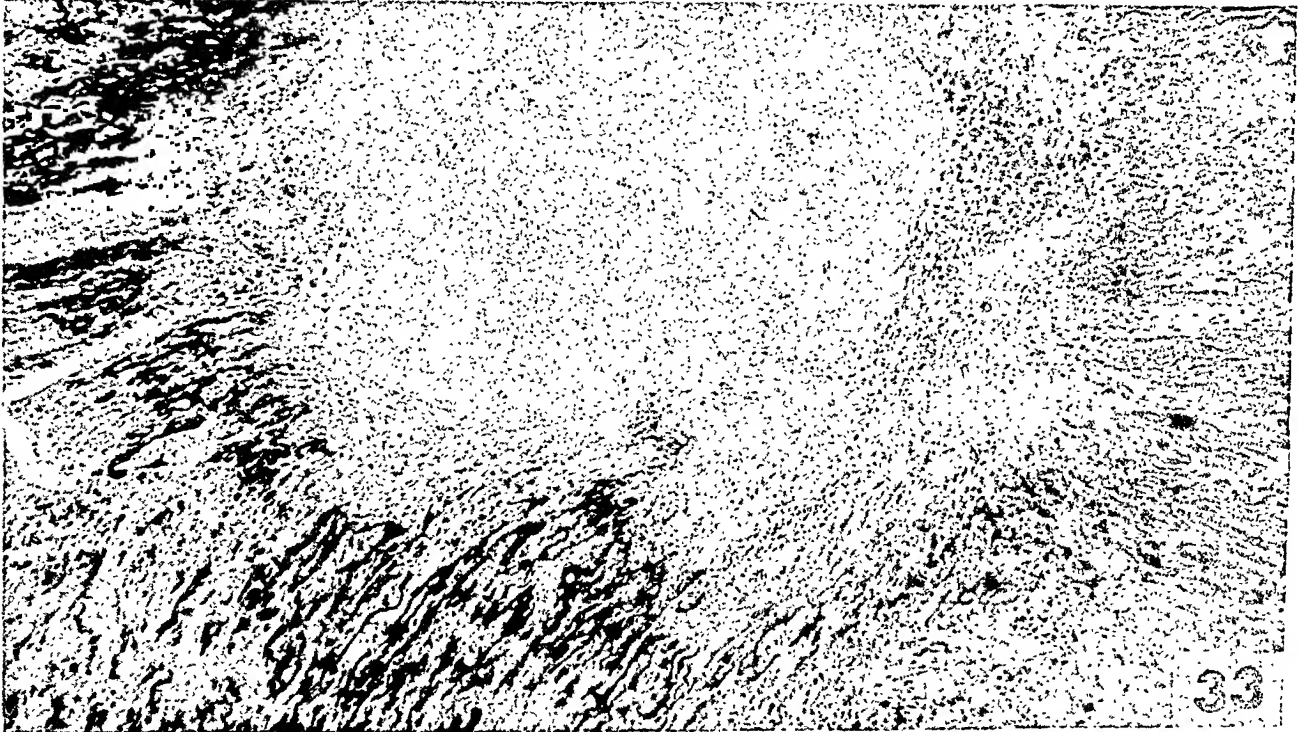
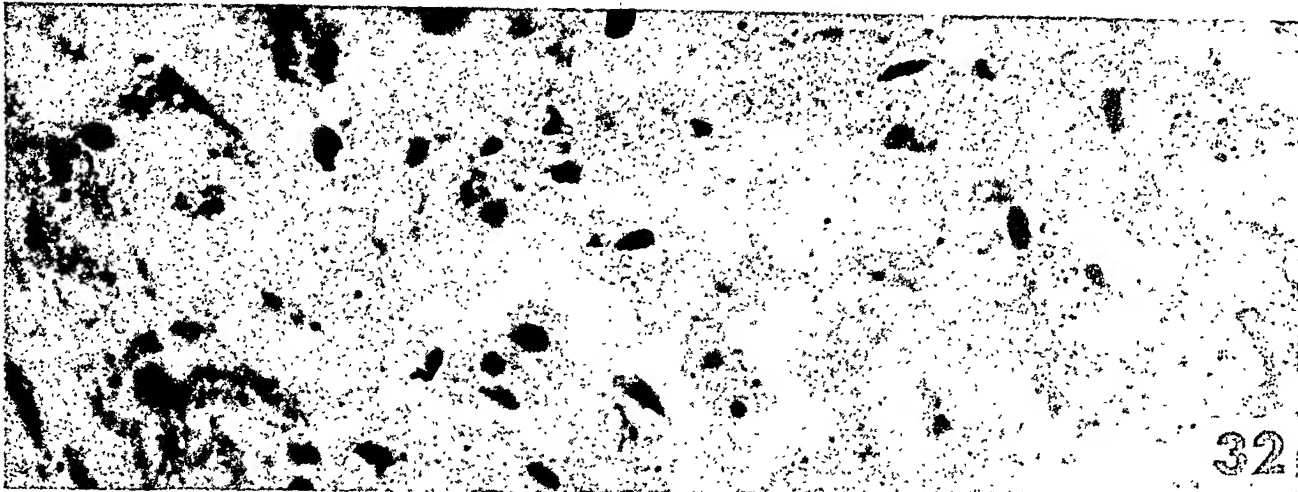
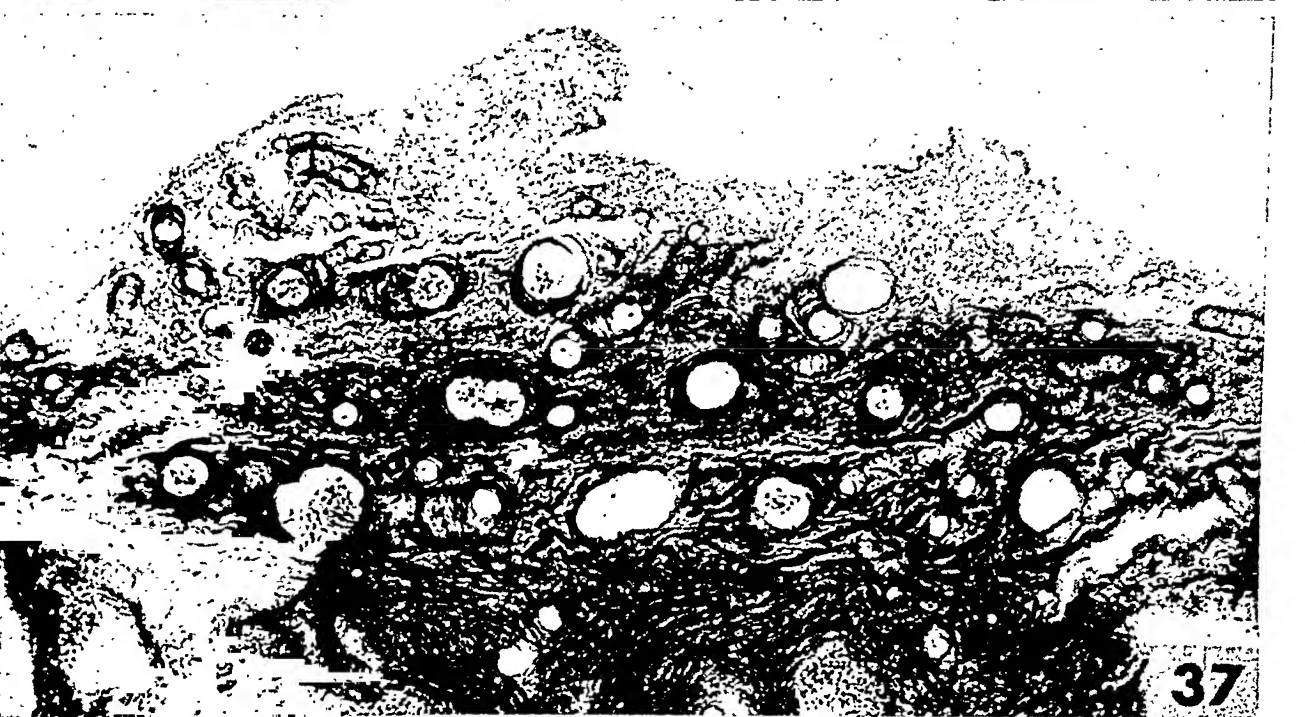


PLATE 15

- FIG. 35. Peribronchial sclerosis. This lesion is due to factors similar to those which produce the typical silicotic nodule, modified by the special disposition of the lymphatic vessels along the bronchial tree. $\times 110$.
- FIG. 36. Thickening of the visceral pleura; blood and lymphatic vessels considerably dilated; hypertrophy of the reticular structure. These lesions show the first stage of silicotic pleuritis. $\times 70$.
- FIG. 37. Pleural silicotic plaque completely constituted. The newly formed capillaries are spread over the surface of the serosa and are accompanied by a very delicate precollagen network. $\times 70$.



TRUE ANEURYSMS OF THE MITRAL VALVE IN SUBACUTE BACTERIAL ENDOCARDITIS *

OTTO SAPHIR, M.D., AND ELIE P. LEROY, M.D.

(From the Department of Pathology,† Michael Reese Hospital, Chicago 16, Ill.)

Mycotic aneurysms of the aortic or mitral valve are not rare in patients with subacute bacterial endocarditis. These aneurysms are usually erosive in character in that they develop from an ulcerative endocarditis. Most commonly they consist of a hollow thrombotic mass attached to the valve. An erosion or ulcer of the involved cusp or leaflet leads into this cavity. To name such aneurysms correctly Ribbert¹ coined the term "thrombo-aneurysma." It is obvious that these are false aneurysms brought about by a destructive lesion in the valve. From the description in the literature of aneurysms found in association with subacute bacterial endocarditis, it is clear that almost invariably they are examples of such false erosive mycotic aneurysms. As early as 1867 Pelvet² was able to collect 9 examples of such aneurysms.

Von Arx³ reported 4 instances of aneurysms of the heart valves. He concluded that they were false aneurysms and denied the existence of true nontraumatic mycotic valvular aneurysms. Ribbert,¹ in 1924, also studied the various mycotic valvular aneurysms but left the question open as to the existence of true valvular mycotic aneurysms. Such aneurysms, Ribbert remarked, would be the result of partial destruction of the valve leaflet, leaving a subendocardial layer intact, with subsequent replacement of the necrotic portion of the valve by granulation tissue. This eventually would give way to intracardiac pressure and form the wall of a true aneurysm.

Kaufmann⁴ (1929) differentiated two types of aneurysms of the heart valves. One type is explained by the destruction of a layer of the valvular endocardium by progressive ulceration. Thrombotic masses and possibly necrotic valvular fragments form a considerable part of the wall of such an aneurysm, being thrust into the sac by the blood pressure. In other instances he asserted that the aneurysmal sac expands slowly until it reaches the size of a walnut, and then may contain thrombi or fluid blood. These latter aneurysms are referred to as chronic aneurysms. His Figure 10 depicts such a chronic aneurysm on the aortic tip of the mitral valve. However, Kaufmann does not distinguish between true and false aneurysms.

Pichon and Bidou,⁵ in 1932, in a very short case report of subacute bacterial endocarditis, mentioned an aneurysm which was situated on

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the aortic leaflet of the mitral valve, bulging towards the atrial cavity. The aneurysm was nodular and disclosed a small point of rupture which was covered by a thrombus. The opening of the aneurysm measured 5 mm. The cavity was filled with clotted blood and its wall was very thin. There is no microscopic description, and there are no clear-cut illustrations. From the description it is not evident whether this was a true or false aneurysm. Soulié and Porge,⁶ in 1937, also described an aneurysm of the aortic leaflet of the mitral valve in an instance of subacute bacterial endocarditis. The aneurysm measured about 3 cm. in greatest diameter, was saccular, and bulged into the left atrium. Its atrial surface was smooth and almost completely covered by endocardium. At the dome of the aneurysm was a small oval perforation which measured 5 mm. in greatest dimension. The aortic valve was retracted and fibrosed. Small vegetations and ulcerations were present on the aortic valve and adjacent to the aneurysm. Microscopic examination of the aneurysm disclosed destruction of the fibro-elastic tissue of the leaflet. It was replaced by inflammatory tissue which was partially necrotic. While from the gross description and the accompanying photographs it is evident that these authors described a true aneurysm, the microscopic description seems too inadequate to allow any definite conclusions as to its nature.

This study is based on the examination of 53 hearts with subacute bacterial endocarditis. Special attention was paid to the presence or absence of aneurysms of the valves. Such aneurysms were encountered in 12 cases. False aneurysms as described above were found in 7, and true aneurysms in 5 patients. All were located on the mitral valve. Only the true aneurysms form the basis of this study.

The diagnosis of subacute bacterial endocarditis was based on the evidence of an old valvular lesion, on the presence of relatively large vegetations, the involvement of the mural endocardium, and the discovery of *Streptococcus viridans* in the circulating blood of the patients at some time before their death. Also the prolonged clinical course of the disease was taken into consideration. The vegetations were rather firm, apparently partially organized, and occasionally areas of calcification were palpated. Though small ulcerations, particularly at the free margins of the involved valves, often were encountered in subacute bacterial endocarditis, they were not associated with true aneurysms. In 4 instances the older valvular lesions were obviously inflammatory, most likely of rheumatic origin. In one instance there was only an old syphilitic aortitis with involvement of the aortic valve. Vegetations, however, were present on both the aortic and mitral valves and the aneurysm was found on the anterior leaflet of the mitral valve.

In 4 instances the aneurysms were located on the aortic leaflet of the mitral valve and in one on the posterior leaflet. In one instance multiple aneurysms were encountered.

The morphologic appearance of these aneurysms was more or less similar. Each presented a well circumscribed, round, smooth out-pouching or ballooning of part of a leaflet of the mitral valve. They measured from about 0.8 to 1.5 cm. in diameter. At first glance they resembled well organized, broad-based vegetations covered by smooth endocardium. Only by gentle compression of the sac-like structure was it possible to recognize the outpouchings as aneurysms. At the point of the highest convexity a small perforation was noted in 4 aneurysms. In only one a small, thin, fibrin thrombus was noted at the point of perforation. In 4 hearts small, firm vegetations were encountered at the periphery, adjacent to the bases of these aneurysms. These vegetations were whitish yellow and were covered by endocardium. In the one heart with several aneurysms they appeared in the form of soft, broad, oval folds with smooth surfaces covered completely by endocardium.

The ventricular surface of the aortic leaflet of the mitral valve was thickened and somewhat retracted in all instances, and, in 4, organizing and organized vegetations covered by endocardium were found. On the ventricular surface of this leaflet, corresponding to the aneurysms which bulged towards the atrium, were small, round defects with smooth but thickened margins. These defects formed the openings into the aneurysms. The defects or entrances into the aneurysmal cavities were much smaller than the aneurysms themselves. When the aneurysms were incised, cavities were encountered containing blood or small thrombi which were only loosely attached to the wall of the aneurysm.

Portions of the aneurysms were examined histologically. A number of sections were cut from the involved leaflet and aneurysmal wall. The sections were stained with hematoxylin and eosin, iron hematoxylin, orcein, and according to the van Gieson method. Also the Giemsa stain was used when deemed necessary.

In the microscopic description the nomenclature of the various layers of the mitral leaflets as used by Gross and Kugel⁷ will be adhered to. The ventricularis of the aortic leaflet of the mitral valve adjacent to the aneurysm disclosed thickened elastic lamellae. In the region of the aneurysm, however, the lamellae were thinned out, and splitting of the elastic fibers was noted. Between them was a cellular connective tissue with a few lymphocytes and endothelial leukocytes. The fibrosa which normally forms the predominate part of the leaflet was severely reduced. The parallel arrangement of the collagenous fibers could not

be made out. Certain fibers were much thicker than is normal while others were replaced by young connective tissue. A moderate number of lymphocytes and endothelial cells were present.

A few newly formed blood vessels were encountered, some of which were dilated to form small blood sinuses. No distinct spongiosa was recognized, the thinned-out fibrosa being covered directly by the auricularis. Whereas normally, as Gross and Kugel⁷ stated, a continuation of the endocardial smooth muscle can usually be traced down the auricularis layer, covered by auricularis elastic lamellae, no evidence of smooth muscle was present in the region of the aneurysm. The elastic fibers were broken, thinned out, poorly stained, and often split into thin fragments which formed irregularly outlined masses of elastic tissue. Inflammatory cells were more numerous in this layer than in other portions of the wall of the aneurysm. A few polymorphonuclear leukocytes in addition to endothelial leukocytes and lymphocytes were encountered. The endothelial layer over the aneurysm was intact. However, the normally present jelly-like zone between the endothelial layer and most superficial elastic fibers could not be seen. At the very tip of the convexity of the aneurysms only a small amount of thin collagenous tissue was seen, covered by endothelium.

Microscopic sections taken from various other portions of the valve disclosed granulation tissue and scar tissue. Organized and organizing vegetations were present. Often granulation tissue was noted at the base of the vegetations while their more distal parts consisted of fibrin with polymorphonuclear leukocytes and much bluish-staining amorphous material. The latter contained no bacteria but gave the staining reaction of calcium salts as was described in a previous study.⁸ Some of the vegetations were completely organized and covered with endothelium.

To summarize the foregoing: In 5 instances of subacute bacterial endocarditis true aneurysms were found on the mitral valve. These aneurysms were definitely not "thrombo-aneurysms" (Ribbert¹), or false aneurysms, but the result of valvulitis with consequent formation of granulation tissue and scar tissue which succumbed to intraventricular pressure with the formation of sac-like outpouchings. Four of the 5 aneurysms had a recent tear at the tip of their convexity.

As stated above, these 5 valvular aneurysms were encountered among 53 patients with subacute bacterial endocarditis. These aneurysms were encountered between 1943 and 1946. In none of 41 hearts with subacute bacterial endocarditis observed between 1935 and 1943 were true aneurysms found. False aneurysms (thrombo-aneurysms) were found 7 times in this period. The 5 true valvular aneurysms were noted

in 5 of 12 hearts with subacute bacterial endocarditis in the autopsy material from 1943 to 1946. In order to find factors which could explain the increased incidence of these aneurysms, the records of the patients presenting these lesions were reviewed and compared with those of previous years. No difference was noted in the early clinical picture of the disease and in the general pathologic changes in the two groups. The organism most frequently found in the blood was *Str. viridans*. However, in those instances in which valvular aneurysms were observed, the patients had received massive doses of penicillin associated sometimes with sulfonamide drugs and heparin. The clinical course of these patients was more protracted and 2 had been pronounced cured. Previous to 1943 the treatment was mainly palliative and symptomatic, and such chemotherapy as was used had been inadequate.

From the histologic examination of the aneurysms it seems clear that the healing process of the valvulitis formed a *locus minoris resistentiae*. The changes were found principally in the zona fibrosa of the valve which normally constitutes its largest part. This region, being replaced by granulation tissue and young scar tissue, succumbed to the intra-cardiac pressure and formed the aneurysms.

Paradoxical as it may seem, these aneurysms thus may be regarded as evidence of healing and indicate at least partially successful treatment of patients with subacute bacterial endocarditis. Libman and Friedberg,⁹ in their monograph on subacute bacterial endocarditis, mentioned the presence of mycotic aneurysms not only in the arterial system but also on the heart valves. They did not classify these aneurysms into true or false aneurysms. However, from their gross description of the aneurysm of the aortic valve as "ballooning of the cusp" and from the accompanying picture it is clear that they found true aneurysms, although there are no microscopic descriptions of them. It is noteworthy that these authors found such aneurysms in the so-called bacteria-free stage of subacute bacterial endocarditis. It seems to us that "bacteria-free stage" implies some progress towards healing. These authors also mentioned an instance of "ballooning of the aortic cusp" in a patient who died 8 years after an attack of subacute bacterial endocarditis. Rosenblatt and Loewe¹⁰ described, in an instance of healed subacute bacterial endocarditis, an aneurysm-like sac in the aorta just above the attached margin of one of the cusps of the aortic valve. The base of the right auricular appendage covered the roof of the aneurysm. Although the authors believed that this aneurysm constituted a congenital anomaly, from the foregoing it seems that this lesion might very well have been a true mycotic aneurysm as here described,

and thus evidence of healing or healed subacute bacterial endocarditis.

In 4 of the 5 patients in this series the aneurysms had ruptured. Since the aneurysms were located on the mitral valve, the rupture obviously caused an insufficiency of this valve or increased a pre-existing insufficiency. From the gross and histologic appearance of these ruptured areas it is obvious that the ruptures must have occurred shortly before death. It is likely that the suddenly increased burden upon the myocardium was a contributing and final factor in the death of these patients.

Modern treatment has altered the mortality rate of subacute bacterial endocarditis. This is due to the fact that the infectious agents of this disease are made innocuous. It has been shown that some of the patients treated successfully as far as the infection was concerned, died some time later because of the concomitant myocardial damage.⁸ This study shows that some of these patients may die as the result of valvular aneurysms incident to the use of the newer therapeutic methods.

SUMMARY

True aneurysms of the mitral valve, mycotic in nature, have been noted 5 times among 12 patients with subacute bacterial endocarditis during the period between 1943 and 1946. These patients had been treated with various sulfonamide drugs, heparin, and penicillin. Among 41 patients with subacute bacterial endocarditis autopsied between 1935 and 1943 there was not a single instance of true aneurysm. Grossly, the aneurysms resembled broad-based, healed vegetations. Only on palpation was their nature recognized. These aneurysms are the result of a circumscribed valvulitis with consequent granulation tissue and young scars which, due to intracardiac pressure, formed sac-like outpouchings. All of these aneurysms were covered by the valvular endothelium. Four of them had ruptured shortly before the death of the patient. The sudden increase of incompetence of the mitral valve was a contributory factor in the death of these patients. These aneurysms are considered as evidence of healing of the valvular lesions in subacute bacterial endocarditis. Their increasing frequency may be attributed to the use of modern therapeutic agents.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

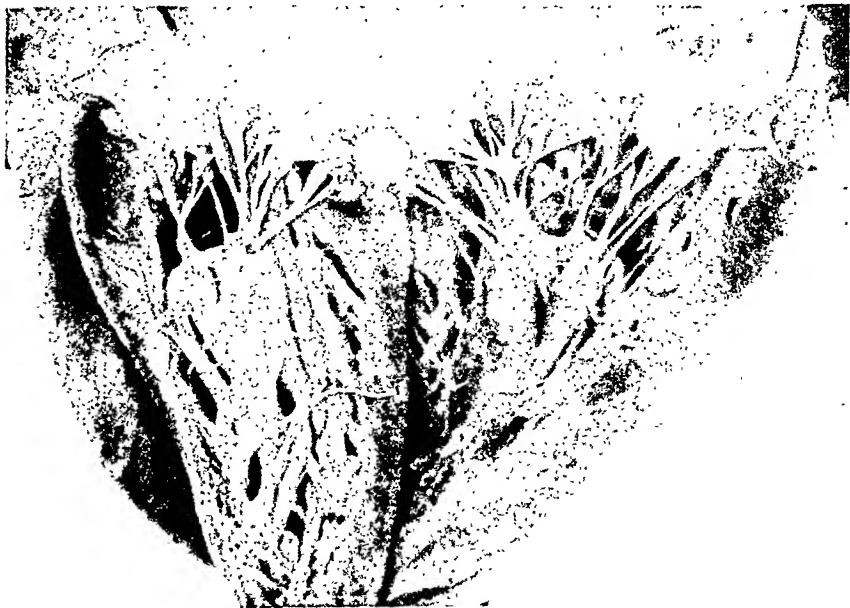
PLATE 16

- FIG. 1. Aneurysm of the mitral valve. The area of perforation disclosed a small thrombus.
- FIG. 2. Aneurysm of the mitral valve. Healing vegetations surround the aneurysm.
- FIG. 3. The aneurysm shown in Figure 2, moderately enlarged.

1



2



3



PLATE 17

FIG. 4. Multiple aneurysms of the mitral valve.

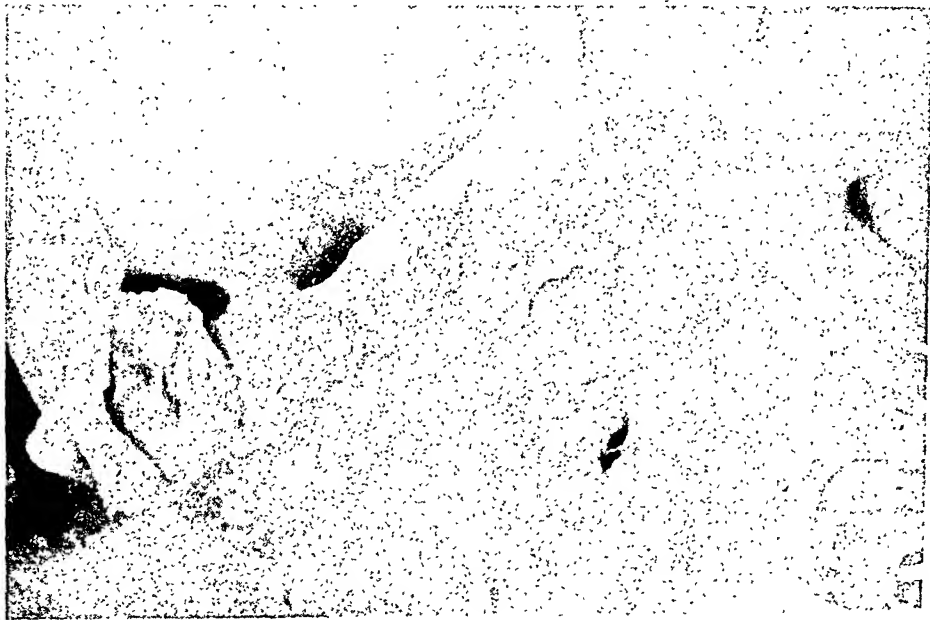
FIG. 5. Ventricular aspect of one of the aneurysms (entrance into the aneurysm).
Of note are the partially organized vegetations just above the opening into the aneurysm and on the aortic valve.

FIG. 6. Ventricular aspect of the aneurysm, showing the smooth margin.

4



5



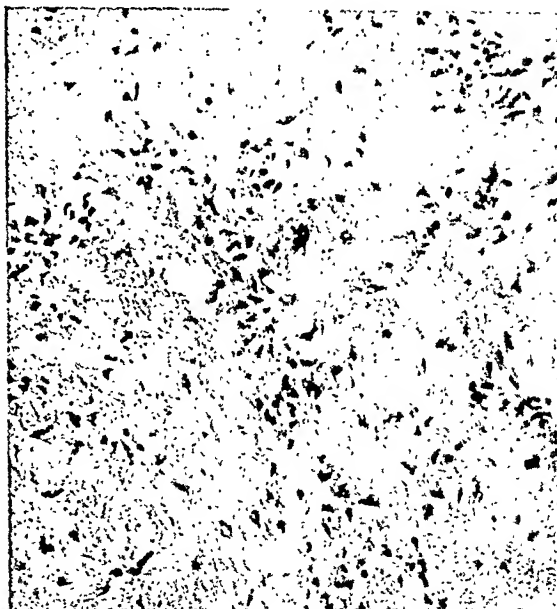
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PLATE 18

- FIG. 7. Section of the mitral valve adjacent to the aneurysm. Of note are the small-sized blood vessels and inflammatory cells. Iron-hematoxylin and eosin stain. $\times 150$.
- FIG. 8. Mitral valve, region of the aneurysm. Granulation tissue is present. Iron-hematoxylin and eosin stain. $\times 300$.
- FIG. 9. Mitral valve, auricular aspect. Of note are the fibroblasts in the region of the aneurysm, and the normal covering by endothelium. Iron-hematoxylin and eosin stain. $\times 150$.
- FIG. 10. Blood sinuses are present in the region adjacent to the aneurysm. Iron-hematoxylin and eosin stain. $\times 150$.
- FIG. 11. Mitral valve, region of the aneurysm, showing splitting of the elastic lamellae and the intact zona auricularis. Iron-hematoxylin and eosin stain. $\times 150$.

7



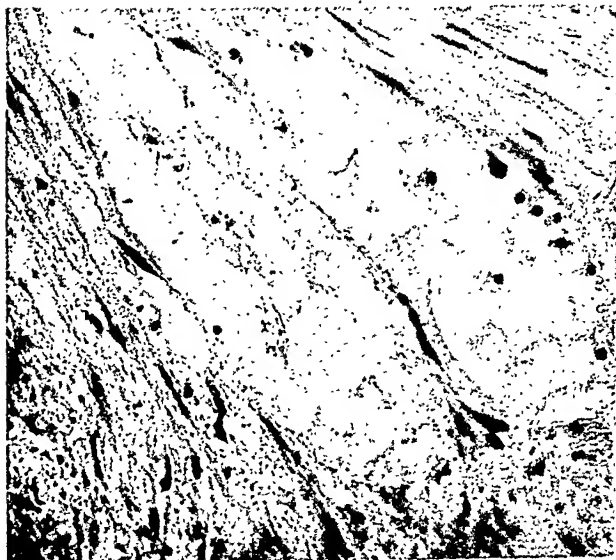
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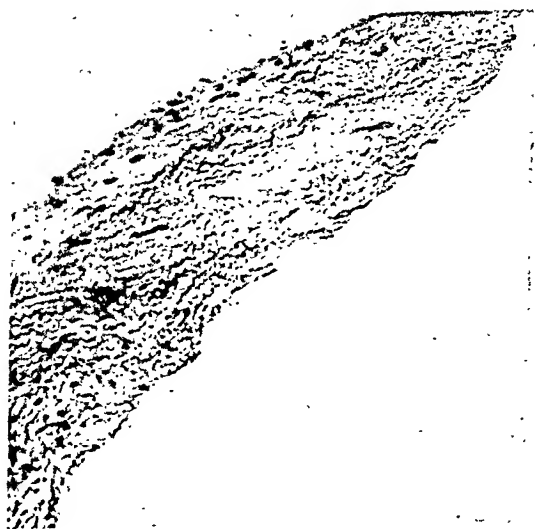
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VIRUS MYOCARDITIS PATHOLOGIC AND EXPERIMENTAL STUDIES *

EDWARD C. H. SCHMIDT, M.D.

(From the Department of Pathology, St. Luke's Hospital, Kansas City, Mo.)

In 1899 Fiedler¹ described isolated myocarditis in man, and a careful review of the literature since that time discloses many reports of this condition in both man and animals. Moreover, a long list of bacterial and other agents have been suggested as etiologic factors in many of these reports. The lesion has been recorded under a variety of names; for example, Fiedler's myocarditis, myocarditis of unknown etiology, allergic, isolated, primary, interstitial, circumscribed, diffuse, and idiopathic myocarditis. The microscopic findings in these recorded cases have stressed polymorphonuclear and mononuclear interstitial infiltration, often with necrosis of myocardial fibers.

Since Fiedler's original description,¹ myocarditis apparently has been produced under variable conditions in several different laboratory animals but without sufficient changes for gross identification. In 1924 Miller² described a myocardial lesion occurring in 60 per cent of 34 rabbits which had been known to be otherwise healthy for periods varying from 2 to 124 days. Microscopically, these lesions consisted of lymphocytes and endothelial leukocytes, and only occasionally were polymorphonuclear eosinophils, plasma cells, and fibroblasts seen. Spontaneous myocarditis with slight inflammatory changes was reported in about 30 per cent of one series of Swiss strain mice.³ Myocarditis has been found in middle-aged mice, between 10 to 20 months of age, accompanied by pleuropericardial hemorrhage and frequently by testicular hemorrhage. Furthermore, inoculations of heart tissue from 7 mice with myocarditis into 74 other mice by various methods were without results, nor could a causative agent be isolated.⁴ Torres⁵ described spontaneous interstitial myocarditis in apparently healthy dogs. The cellular infiltrations were chiefly perivascular in distribution and were composed of lymphocytes, macrophages, and ameboid wandering cells. Other investigators have described myocarditis in animals occurring in cases of both mineral and protein deficiencies.^{6,7}

In 1942, French and Weller⁸ described interstitial myocarditis occurring in human beings, rabbits, rats, and mice following the use of neoprontosil, sulfanilamide, sodium sulfapyridine, and sodium sulfathiazole. The cellular infiltration of this lesion consisted of large mononuclear cells of clasmatocytic type and numerous cells with granular

* Received for publication, April 7, 1947.

eosinophilic cytoplasm, a few of which were polymorphonuclear. Experimentally, they produced the disease in 38 of 60 mice and in 33 of 40 rats. Spontaneous myocarditis occurs occasionally in rabbits, and Pearce⁹ was able to produce myocardial lesions consistently in rabbits by introducing a solution of acacia immediately prior to inoculation of vaccine virus, the myxoma virus, or two strains of the fibroma virus. He obtained cardiac lesions with necrosis and an inflammatory reaction which differed only slightly from virus to virus. In 1945 Finland and co-workers¹⁰ isolated the influenza A virus from the lungs of 2 patients dying of interstitial myocarditis. The heart fibers showed focal necrosis and invasion by large mononuclear cells. The interstitial tissues were infiltrated with large mononuclear cells, lymphocytes, plasma cells, eosinophils, and polymorphonuclear leukocytes. In 1945 Helwig and Schmidt¹¹ isolated a virus from a group of anthropoid apes dying from interstitial myocarditis and produced myocardial lesions consistently in mice. This report is concerned with further investigations and analysis of this virus.

ISOLATION OF THE AGENT

While studying the causes of death of the animals at the Anthropoid Ape Research Foundation at Dania, Florida, 3 cases of interstitial myocarditis were encountered. The first occurred in November, 1944, in an adult male gibbon. This animal had been placed in a prominent cage in the farm where many visitors were in fairly close contact with it for several weeks prior to its demise. However, for 10 days before death it had been fairly well sheltered from visitors. No signs of illness were observed until death occurred suddenly and unexpectedly. Autopsy revealed a dilated heart, a slightly blood-tinged pericardial effusion, bilateral hydrothorax, and marked pulmonary edema. An intense interstitial myocarditis was found on histologic examination. No gross or microscopic lesions were found in the brain. The spinal cord was not examined. No attempt was made to recover the etiologic agent.

The second case was encountered in February, 1945, in a well developed, well nourished, 5-year-old male chimpanzee. This animal had been displayed at the entrance to the farm where it was in close contact with visitors, many of whom petted the animal. One morning this chimpanzee vomited its breakfast and died suddenly. Late in the afternoon the body was placed in a refrigerator at about 40° F. The following morning it was examined. The pertinent findings were massive bilateral hydrothorax with slightly pink but clear fluid, distention of the pericardial sac with clear fluid, wide dilatation of all chambers of the heart, and marked bilateral pulmonary edema. Microscopic examination of the heart revealed many minute foci of necrosis with an infiltra-

tion of lymphocytes and scattered polymorphonuclear leukocytes, and pyknotic nuclear structures. Chest fluid and a saline suspension of ground spleen were inoculated into mice and the virus was thus recovered.

The third case of anthropoid myocarditis was found during October, 1945, in a 6-year-old female chimpanzee which had been on exhibition in the same location as the second case. This animal appeared ill for a period of 5 days with edema of the face, subnormal temperature, and dysphagia, and terminally showed considerable cyanosis and dyspnea. Forced feeding and intravenous fluids failed to alter the gradual decline of the animal to its death.

The body had been refrigerated for more than a day at the time of examination. The heart was flabby, weighing 200 gm., the right ventricle was markedly dilated, and on section many grayish discolored, semi-softened areas were present in the myocardium of both ventricles. Bilateral hydrothorax of 500 cc. on the right and 550 cc. on the left, and a marked hemorrhagic pulmonary edema were present. Microscopic examination of the heart showed extensive necrosis and diffuse round-cell infiltration. In many areas the individual fibers were fragmented or entirely replaced by the inflammatory infiltration consisting of lymphocytes, mononuclear wandering cells, and polymorphonuclear leukocytes. The brain and spinal cord revealed no gross or microscopic abnormality. Mice inoculated with this pleural fluid failed to develop any of the typical lesions.

The etiologic agent was recovered from the second case by inoculating two groups of 5 mice, one group with chest fluid and the other with spleen suspended in saline solution. All 10 animals died following paralysis of the hindquarters. A suspension of lungs, hearts, and spleens from these animals was inoculated into 10 other mice intravenously and intracranially. These animals were all paralyzed on the 4th day and dead by the 8th day. These two series of experiments were repeated by inoculation with the original fluids, and the organs from the second series suspended in saline solution formed the basic inoculum with which all subsequent studies were conducted. The virus from the second group was passed through 10 successful mouse passages before it appeared to lose its potency to any degree. The mice all followed a very characteristic course manifested by paralysis on the 4th or 5th day and death on the 7th to 10th day.

TYPES OF LESIONS

In order to study the chronologic development of the disease, a group of 26 mice was inoculated simultaneously and the tissues studied

microscopically at various intervals. The animals appeared quite well for 2 days; then on the 3rd day many developed rough coats. On the 4th and 5th days approximately one-half developed paralysis of the hind legs and by the 9th day all except 2 had developed paralysis. The paralysis increased in severity until the mice lost all function of the hindquarters and dragged them behind the rest of the body, as in the paralysis caused by the Theiler virus.¹² Finally most of the trunk muscles were involved. In early experiments most animals died by the 8th day. In this series 9 were dead by the 8th day and some lived as long as 37 days before being sacrificed. The cardiac lesion varied with the period of inoculation. The earliest microscopic change was a mild perivascular monocyctic infiltration in the heart muscle. About the 6th day small necrotic foci appeared in the myocardium with monocyctic infiltration of the surrounding areas. The muscle fibers were separated by edema fluid and cellular infiltrates. Three days later, in addition to the early inflammatory changes, necrosis became more extensive and fibroblasts began to appear. In small foci the muscle was entirely replaced by mononuclear cells and fibroblasts. This was the usual condition of the myocardium in mice succumbing to the virus. Animals sacrificed or dead on the 9th or 10th day frequently showed marked involvement of the auricles. Hearts of animals which survived to the 11th or 12th day had lymphocytes and fibroblasts in increasing numbers. The heart muscle in a period from the 8th to 12th day was extensively involved, frequently throughout the whole myocardium. Calcification was seen as early as 10 days and was very common after the 12th or 13th day, appearing as small granules in the necrotic foci. These granules of calcium usually were arranged in the pattern of the necrotic muscle fibers and frequently appeared while an acute inflammatory reaction was still in progress. After 20 days fibroblastic replacement and calcification were often the only remnants of the disease. It was interesting to note that if a mouse survived to the 12th day, recovery was the rule.

The same inoculum produced a somewhat different process in guinea-pigs. Thirteen healthy mature guinea-pigs were injected with 0.1 cc. of egg inoculum and sacrificed at the indicated periods. Most escaped clinical stigmata of the disease entirely; only one died and that death followed delivery of a small fetus. Seven showed advanced microscopic myocardial changes and 4 had slight or questionable infiltrates. The earliest lesion was seen at 4 days and minute changes were seen as late as 92 days. In temporal spread, the heart lesions were much more persistent and prolonged than in mice. Whereas the acute phase rarely was seen after 6 to 18 days in mice, it often was seen as late as 30 to 35

days in guinea-pigs. The microscopic picture of the heart was similar to that in mice. Foci of necrosis were not as marked but the inflammatory infiltration of the muscle was as widespread and calcification of muscle fibers as distinct.

Hamsters were susceptible to the virus and developed a disease similar to that seen in mice. Four hamsters were inoculated with mouse organs suspended in saline solution. In 10 days they had rough coats, paralysis of the hind legs, and appeared quite ill. Despite the apparent severity of the disease none died and only 2 showed myocarditis when sacrificed following recovery from the paralysis. Two attempts to pass the virus from these hamsters to other hamsters were unsuccessful.

Rabbits were not very susceptible to the virus. Twenty-one rabbits were inoculated with the original chest fluid, a suspension of original ground spleen, various suspensions of mouse organs and egg inocula. Two of the rabbits which were inoculated with the original chest fluid had myocardial changes. One showed areas of necrosis and infiltration with polymorphonuclear leukocytes and large monocytes; the other showed granular degeneration of the myocardial cytoplasm. Three other rabbits had slight perivascular lymphocytic accumulations but exhibited no definite myocardial changes. One rabbit had lipomatosis destruens of the heart after paralysis of the hindquarters which varied in intensity and finally cleared. Detailed microscopic study of the central nervous system did not reveal any change to account for the paralysis. Eleven of these rabbits had serial electrocardiograms. Several showed transient T-wave changes, but no persistent defects could be followed.

Two groups of rats were injected with different potent inocula without the development of either paralysis or cardiac lesions.

LESIONS OF OTHER ORGANS

The cardiac lesions received special attention in this study and were taken as the criterion of activity of the virus. However, lesions of organs other than the heart were encountered occasionally. The brain, liver, and lungs were examined routinely. The spleen and kidneys also were sectioned in a majority of the cases. Of the organs other than the heart, the central nervous system was affected most frequently. Myelitis was most marked in the period from 4 to 10 days following inoculation when the paralysis was most severe. Sections through the spinal cord at this time showed extensive destruction of all of the outer neural elements with invasion of mononuclear cells and a few polymorphonuclear leukocytes which frequently clumped beneath the meninges. The ganglion cells apparently were not involved by the ex-

tensive myelitis. In this period and somewhat later, foci of encephalomyelitis could be seen in the brain. These areas were frequently beneath the meninges and the outer portion of the brain where they were manifest by groups of mononuclear cells. Frequently there was perivascular cuffing of adjacent vessels. No encephalitis was encountered in the guinea-pigs.

The lungs revealed a variety of minor lesions. Hyperemia was encountered occasionally and often it was accompanied by pulmonary edema which was quite irregular in its distribution. A round-cell thickening of the intra-alveolar septa was encountered and a very small

TABLE I
Total Injections

	Ascitic	Saline	Ascitic-saline	Serum-saline	Glycerinated saline	Other*
Mice	73	52	53	34	14	28
Guinea-pigs	15		21	2	4	2
Rabbits	7	7	5	2	2	

* This group includes several animals which were inoculated with pleural fluid and tissue fluid.

proportion of the animals showed bronchopneumonia in addition to the interstitial pneumonic reaction. This interstitial reaction with edema was seen also in guinea-pigs within 3 weeks of inoculation but was not seen later. Occasionally the spleen was grossly enlarged to two or three times the size encountered in control animals. Microscopic examination of the spleen often showed hyperplasia of the lymphoid follicles. A few mice and guinea-pigs had small foci of round cells collected in the medullary portion of the kidneys and interstitial nephritis was seen in 2 guinea-pigs. The livers occasionally showed moderate venous congestive changes and rarely cloudy swelling was present also.

VIRULENCE OF THE AGENT

Media

Several different media were used to store the virus (Table I). The isolation was accomplished with saline suspensions; however, over a period of 10 days' storage the saline suspensions lost some of their potency. Berkefeld or Seitz filtrates in ascitic or 10 to 50 per cent saline-ascitic mixtures retained potency longer than the saline suspensions. A 10 per cent saline-serum mixture proved as effective as ascitic combinations. Buffered glycerinated saline solution proved an effective carrier in a limited number of inoculations. A summary of inoculations in mice follows:

Nineteen expected potent ascitic suspensions produced myocarditis in 17 groups.

Ten expected potent saline inoculations produced myocarditis in 8 groups.

Eight serum-saline expected potent inoculations produced myocarditis in 8 groups.

Six expected potent ascitic-saline inoculations produced myocarditis in 6 groups.

Three glycerinated saline expected potent inoculations produced myocarditis in 3 groups.

Six expected nonpotent inoculations produced no myocarditis.

Controls

Numerous control groups were studied. Following the isolation of the virus, organs from 5 other anthropoids were inoculated into mice in the same manner used in isolating the agent. Five groups of 4 mice each were inoculated. None of these developed paralysis nor were any myocardial changes noted at autopsy. Two more groups of mice were inoculated with ground heart suspensions from 2 patients, one dying of acute interstitial myocarditis and the other of rheumatic myocarditis. These animals did not have any demonstrable lesions when sacrificed. Three groups were injected with sera from persons suspected of having influenza without the development of any signs of illness or lesions at autopsy.

Various mice were examined at random from the colony and myocarditis was not encountered in any uninoculated animals.

CHARACTER OF THE AGENT

The nature of the agent became apparent when a suspension of it could be passed through Seitz and Berkefeld N filters without loss of potency. Following the second passage through mice, attempts were made to culture organisms from the suspension following filtration. The inocula from 3 successive passages were cultured on all ordinary media for bacteria and yeasts. All of these proved sterile. Only a few passages were made without the virus being passed through a Berkefeld N candle, a Seitz filter, or a Swinney modification of the Seitz filter.

A suspension of mouse hearts in serum-saline solution heated to 70° C. for 20 minutes and potent amniotic fluid heated to 70° C. for 20 minutes were injected intravenously and intraperitoneally into 2 groups of 4 mice each. Neither of these groups developed characteristic lesions. A third group of 6 mice was injected, 2 intravenously, 2

intraperitoneally, and 2 intracranially, with potent ascitic saline suspension heated to 56° C. for 20 minutes. One inoculated intravenously and one inoculated intraperitoneally developed transient paralysis from the 4th to the 6th day. On the 8th day all were paralyzed. None died and at autopsy only 2 had cardiac lesions and these were only moderate.

Due to the limited facilities at hand, the material was stored in a refrigerator freezing unit which maintained a fairly constant temperature at -19° C. The agent retained its potency when stored in the various media for periods as long as 2 months, as indicated by the development of paralysis, myocarditis, and encephalitis following inoculation. Longer periods could not be determined because of mechanical difficulties.

The routine methods of inoculation were intravenously at the base of the tail or intraperitoneally. Intracranial inoculations were used frequently at the beginning of this study, but as they caused rapid paralysis and death before the cardiac lesion was fully developed, other portals seemed more suitable. Nine mice were inoculated by applying the inoculum to the nasal orifices and forcing the mice to breathe through the material. All of these mice showed the most advanced myocardial lesions, following a typical course with paralysis. Eight other mice were inoculated in the same manner except that nasal washings from a paralyzed mouse were used to carry the virus; 5 of these developed cardiac lesions, while 3 showed no clinical signs of the disease.

The ability of the agent to develop immune bodies was demonstrated by a positive protection test using the sera of mice recovered from paralysis combined with potent virus at the time of inoculation. Sera from 2 rheumatic fever patients afforded no protection in 2 mouse protection tests. Yellow fever vaccine injected 2 weeks prior to inoculation provided no protection for 6 mice.

The virus was apparently widespread since it could be transmitted by suspensions of brain, heart, kidney, and spleen, or by nasal secretions. Moreover, its potency did not vary markedly from organ to organ.

The allantoic sacs of chicken eggs were used in several passages. One group of 4 7-day-old eggs opened on the third day following inoculation and passed through a Berkefeld filter proved to be the most potent inoculum. This suspension was used extensively. It produced lesions in mice regularly, but could not be successfully transmitted through eggs again. Two other groups of 7-day-old eggs were successfully inoculated with development of potent allantoic fluids. Five other such groups did not develop the virus in a transmittable form.

COMMENT

Isolated myocarditis is described by Saphir¹³ in an exhaustive and admirable review as exhibiting more or less diffuse inflammatory changes in the myocardium of wide variety and of various causes, having in common principally an isolated involvement of the myocardium by a nonspecific lesion without inflammatory change of the endocardium or the pericardium. The myocardial lesions described by various observers as interstitial myocarditis differed from animal to animal and from one etiologic agent to another. The lesions noted by these authors varied from round-cell infiltration, often perivascular in distribution, to advanced inflammatory reactions with necrosis and polymorphonuclear infiltration.

The myocarditis produced by the virus isolated by us¹¹ from a chimpanzee varied considerably during different stages of the illness. In mice the first stage observed was slight myocardial perivascular round-cell infiltration, which occurred at about the same time as the onset of paralysis. In the period from 6 to 10 days after inoculation, necrosis of heart muscle and polymorphonuclear infiltration appeared. Calcification appeared at 12 to 14 days with a patchy distribution of fine granules, which seemed to coalesce in the later stages when fibroblastic replacement of the myocardium was developing. The widespread interstitial reaction, occasionally extending from the endocardium to the pericardium and often occurring in both auricles and ventricles, was a characteristic reaction with the chimpanzee agent. This agent also had a primary or secondary affinity for the central nervous system in the smaller rodents, and myelitis of the spinal cord was a consistent finding in paralyzed mice.

Warren and Smadel¹⁴ have maintained the virus by intracerebral inoculations and have found it highly neurotrophic. In mice, encephalitis developed rapidly and some died within 24 hours. The agent was quite potent when inoculated either intraperitoneally or intracerebrally in extreme dilutions. As in my experiments, myocarditis was not observed in the rapidly dying animals but extensive focal necrosis was found when the illness ran a more prolonged course. The guinea-pigs inoculated by Warren and Smadel did not develop myocarditis but showed only a febrile reaction following intracranial inoculation. Guinea-pigs, rabbits, and a rhesus monkey developed specific antibodies during convalescence. Attempts to establish immunologic relationships between this virus and 15 known viruses were unsuccessful in their hands.

In my experiments, the site of inoculation, with the exception of the intracranial route, did not appear to affect the lesion produced by this

virus. That the method of inoculation possibly affected the localization of the lesion is further suggested by the fact that myocarditis was produced in 17 of 22 guinea-pigs in my series inoculated intraperitoneally and intravenously, whereas it was absent in those inoculated intracranially by Warren and Smadel.¹⁴

A major concern in my early experiments was that perhaps the inoculations had activated a latent virus in the mouse colony, since the paralysis seen in these animals resembled that described by Theiler¹² in spontaneous encephalomyelitis of mice. However, it was suggested that these were different agents because of the fact that the chimpanzee agent could be transmitted by many different methods and not only by the intracranial and intranasal routes as was observed with the Theiler virus, and also because of the rapidity with which paralysis developed in my animals. Later experiments at the Army Medical Center showed that there was no demonstrable immunologic relationship between the chimpanzee agent and Theiler's virus.

Despite the ready passage of the chimpanzee agent into mice when inoculated by a variety of methods, neither myocarditis nor encephalitis was seen in any control animal. In 9 injected groups, a control uninjected mouse was placed in the same cage with the experimental mice inoculated with the agent. None of these showed signs of disease, nor were any microscopic lesions found in these animals at necropsy. Thirty-nine other mice, which were either inoculated or injected with nonpotent material, did not develop any stigmata of the disease.

The isolation of an agent from an anthropoid ape gave rise to the belief that perhaps this was the etiologic agent of interstitial myocarditis in man. This impression was somewhat strengthened when a member of our laboratory personnel developed signs and symptoms of cardiac disease with an elevated sedimentation rate and slight transient electrocardiographic changes. The electrocardiographic tracing reverted to normal in 2 days and the sedimentation rate decreased slowly. However, other members of the laboratory who were subjected to more extensive exposure remained healthy. Neutralization tests performed on 4 people handling these sick anthropoids and working with the virus were negative. One chimpanzee, which had been next to a chimpanzee with myocarditis, showed a small amount of neutralization antibody. This suggests that the chimpanzee agent has little or no pathogenicity for man.

SUMMARY

1. An agent was isolated from a chimpanzee dying of interstitial myocarditis which produced myocarditis and encephalitis in mice and hamsters and myocarditis in guinea-pigs.

2. This virus produced a range of cardiac lesions from slight perivascular lymphocytic infiltration to advanced myocardial necrosis and polymorphonuclear infiltration.

3. The agent was found to be potent and specific when introduced intravenously, intraperitoneally, subcutaneously, intracranially, and by intranasal instillation. It seemed to be widespread in various organs since it could be transmitted by suspensions of a variety of viscera.

4. This virus has not been identified with any of a variety of known viruses by various biologic tests.

5. The morphologic findings in the heart in this disease duplicate to a remarkable degree the myocardial lesions found in human heart muscle in several virus diseases in patients in whom clinical manifestations were observed prior to death.

Recently Smadel (Smadel, J. E., Medical Department Symposium, Army Medical Center, Washington, D.C., June 5, 1947) reported that stored frozen sera collected from a group of soldiers with "three day fever" in Manila in December, 1945, gave positive results in neutralization tests for this agent. During the course of the illness the antibody titer increased and was highest during convalescence.

I wish to thank Dr. Ferdinand C. Helwig for his guidance and suggestions, and the United States Army Institute of Pathology for the photomicrographs.

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DESCRIPTION OF PLATES

PLATE 19

- FIG. 1. Heart of an adult male gibbon. There is widespread involvement of the myocardium with areas of necrosis. Hematoxylin and eosin stain. $\times 110$. (Army Institute of Pathology accession no. 145645; neg. 89608.)
- FIG. 2. Higher magnification of a field from Figure 1. Hematoxylin and eosin stain. $\times 350$. (A.I.P. acc. 145645; neg. 89609.)

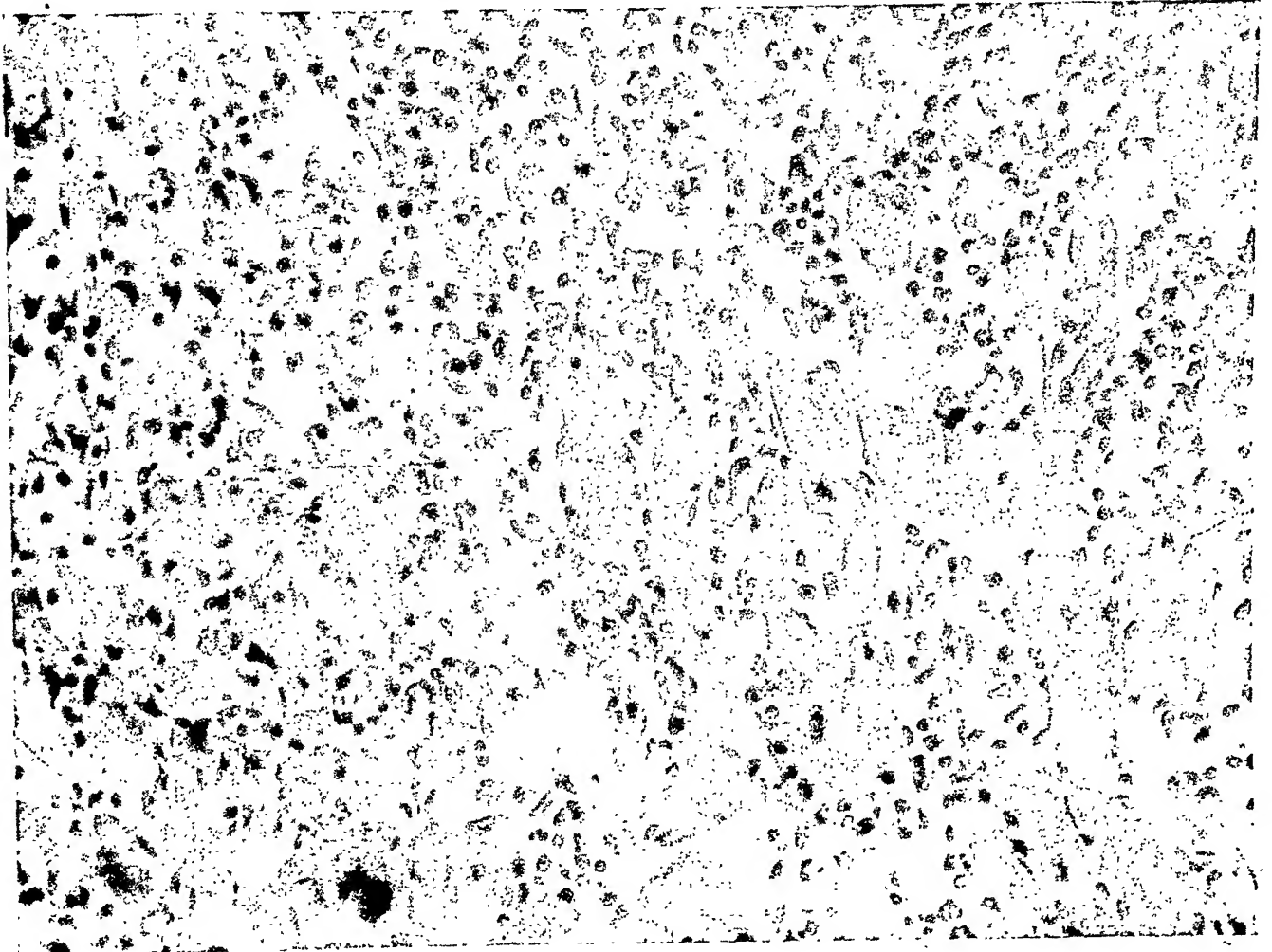
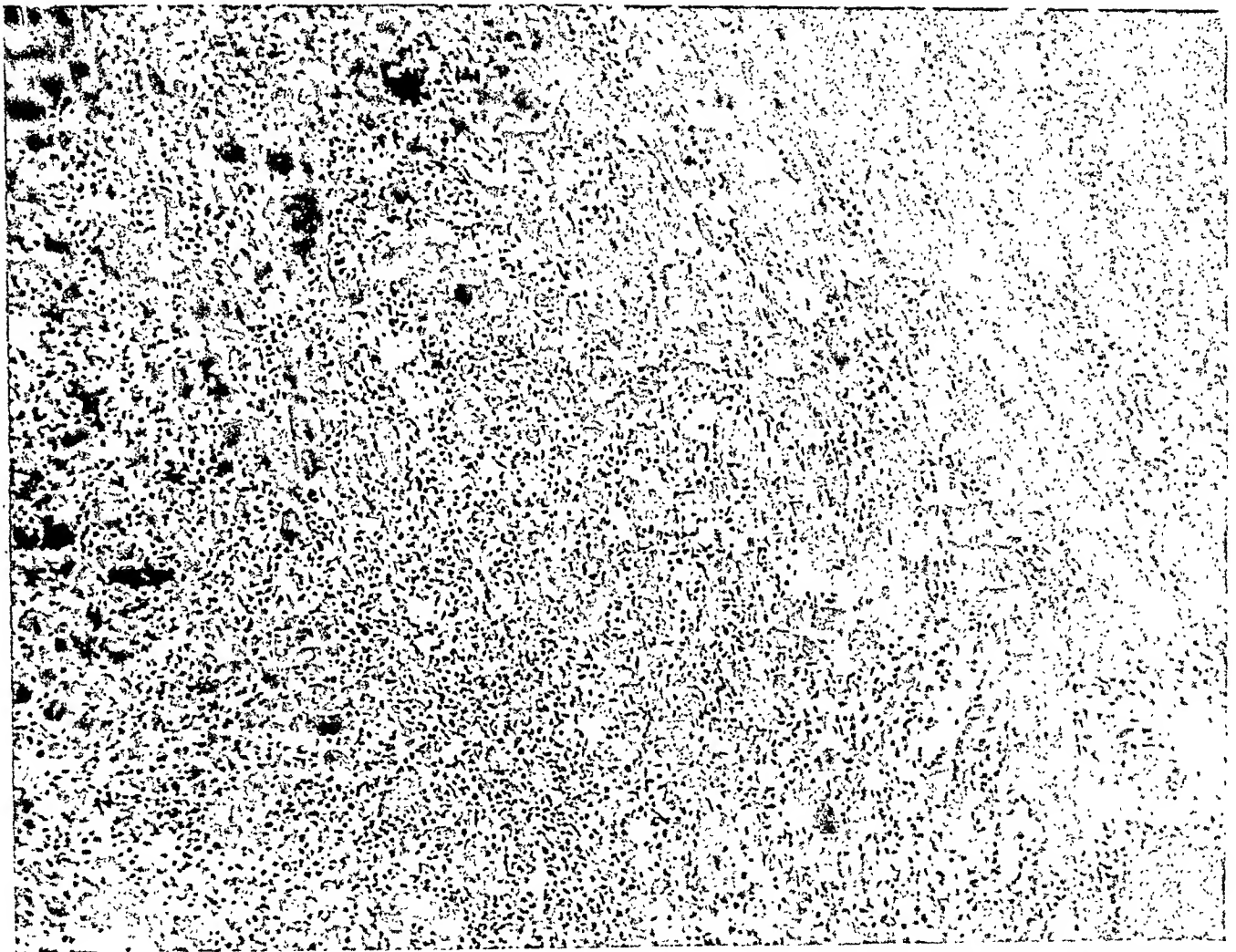
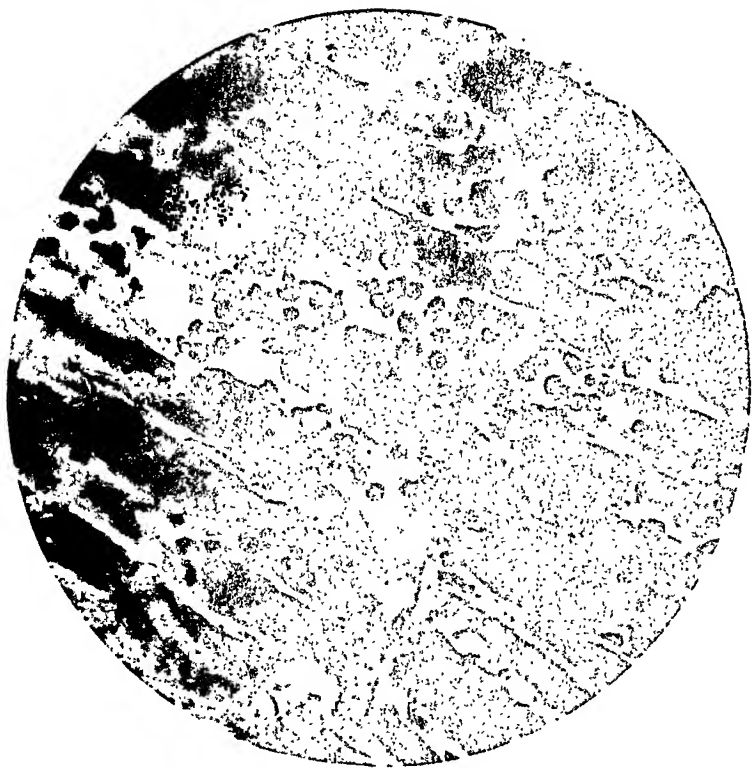


PLATE 20

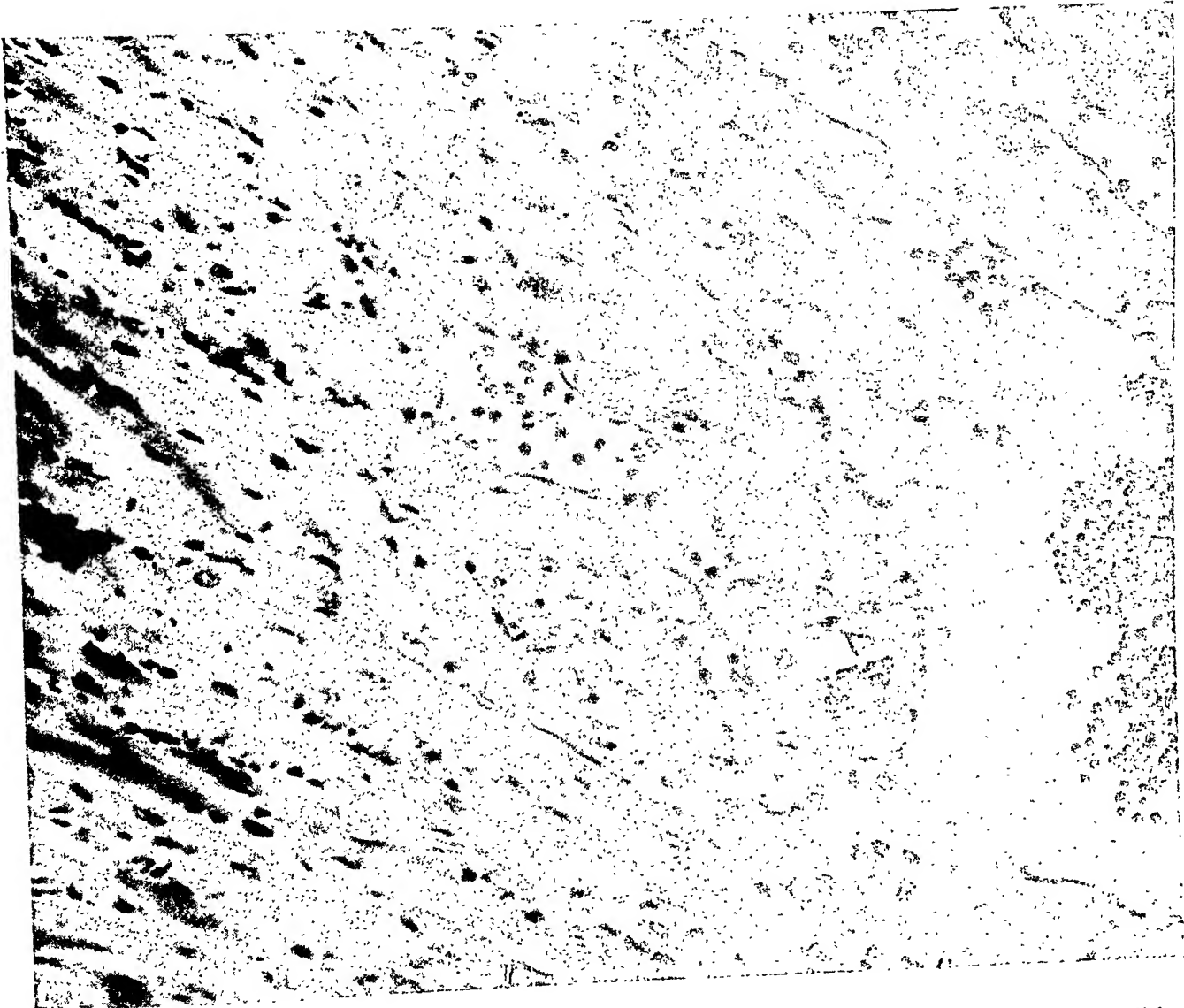
FIG. 3. Five-year-old male chimpanzee. The myocardium is invaded with lymphocytes. Hematoxylin and eosin stain. $\times 380$.

FIG. 4. Mouse heart on the 6th day of inoculation. Edema and round-cell infiltration are present. Hematoxylin and eosin stain. $\times 350$. (A.I.P. acc. 145645; neg. 89603.)

3



4



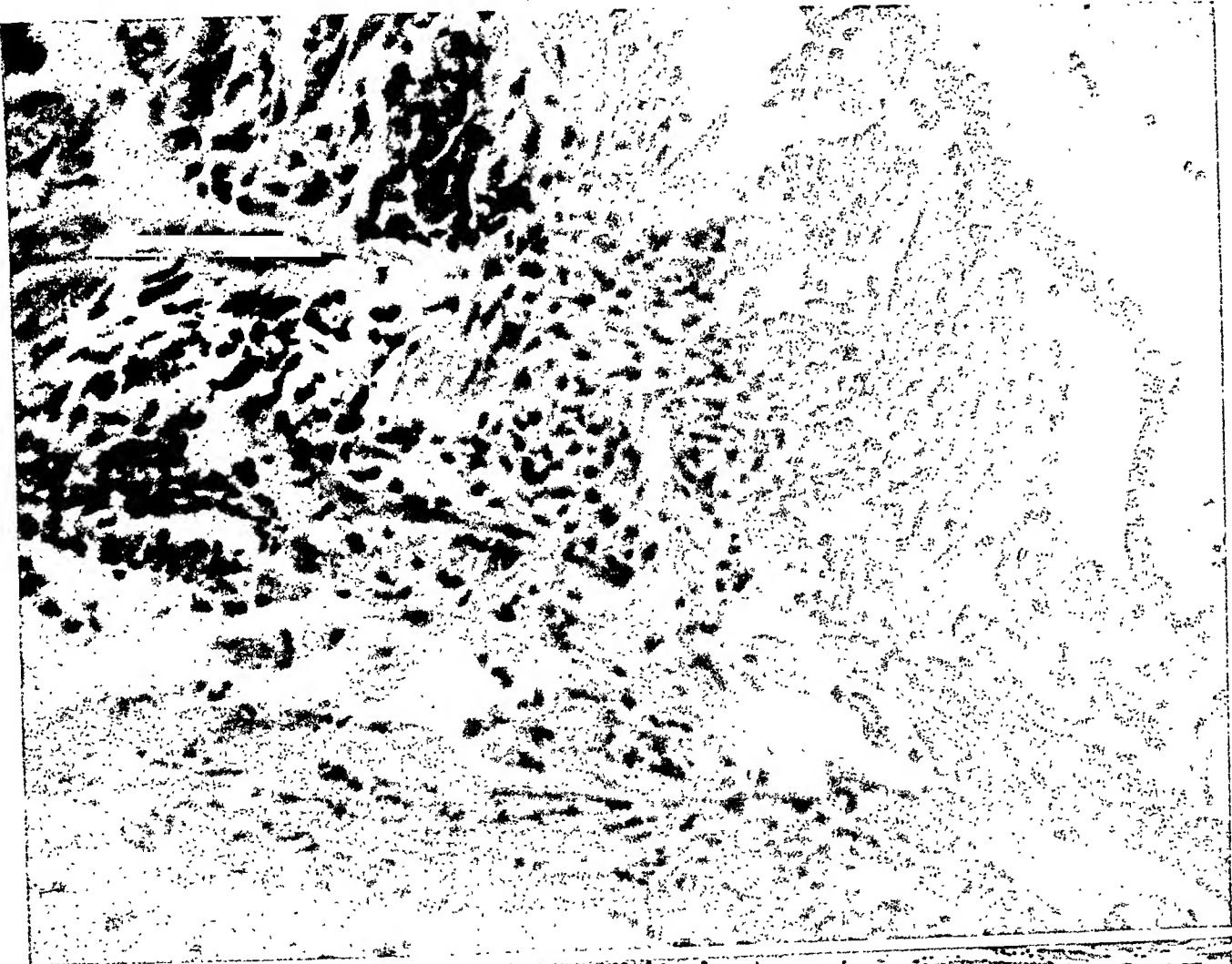
Virus Myocarditis

Schmidt

PLATE 21

- FIG. 5. Mouse heart on the 10th day of inoculation with mononuclear infiltration and early fibroblastic replacement. Hematoxylin and eosin stain. $\times 350$. (A.I.P. acc. 145645; neg. 89601.)
- FIG. 6. Mouse heart on the 10th day following inoculation, illustrating the marked auricular involvement at this stage. Hematoxylin and eosin stain. $\times 110$. (A.I.P. acc. 145645; neg. 89602.)
- FIG. 7. Mouse heart on the 12th day following inoculation. Many fibroblasts are present. Hematoxylin and eosin stain. $\times 110$. (A.I.P. acc. 145645; neg. 89647.)

5



6



7

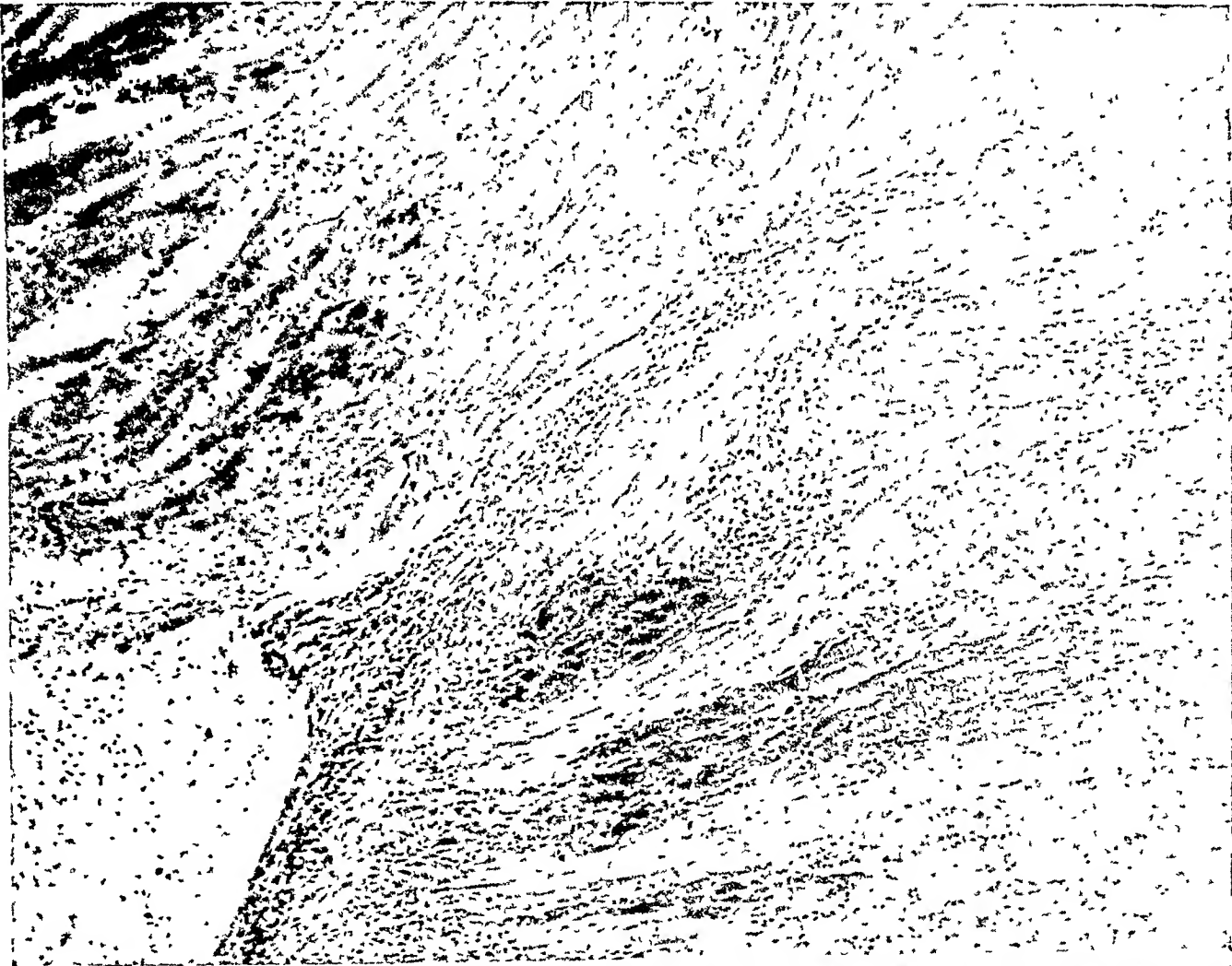


PLATE 22

FIG. 8. Mouse heart on the 13th day following inoculation. Calcification of the necrotic muscle fibers has taken place. Hematoxylin and eosin stain. $\times 110$. (A.I.P. acc. 145645; neg. 89600.)

FIG. 9. Guinea-pig heart 17 days following inoculation. The marked calcification is characteristic of the reaction in this animal. Hematoxylin and eosin stain. $\times 350$. (A.I.P. 145645; neg. 89593.)

8



9

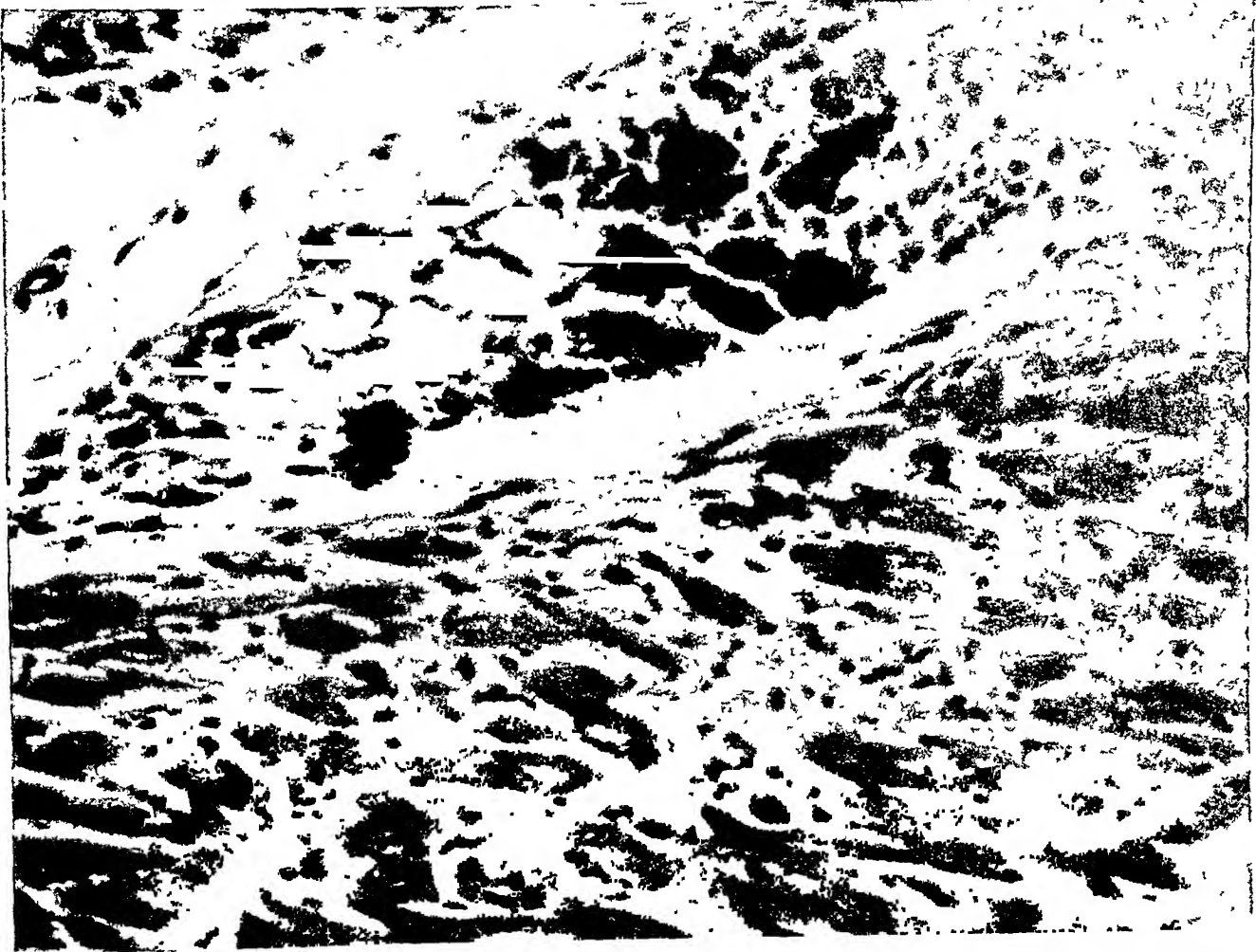


PLATE 23

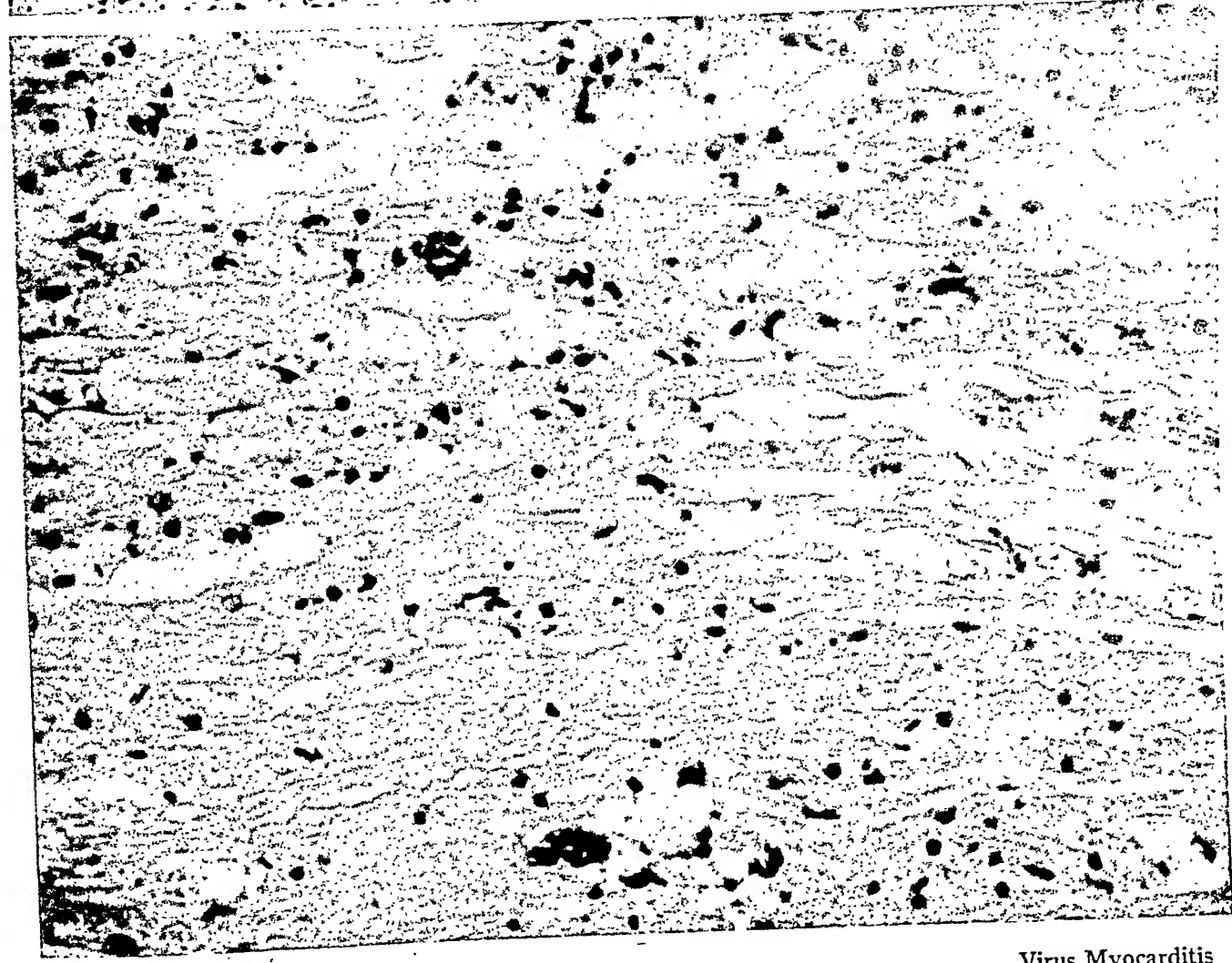
FIG. 10. Spinal cord of a mouse which died on the 10th day following inoculation. The peripheral myelitis with perivascular cuffing is evident. Hematoxylin and eosin stain. $\times 110$. (A.I.P. acc. 145645; neg. 89649.)

FIG. 11. Higher magnification of a field from Figure 10. Hematoxylin and eosin stain. $\times 350$. (A.I.P. acc. 145645; neg. 89648.)

10



11



Virus Myocarditis

HISTOLOGIC CHANGES IN RICKETTSIALPOX *

VERA B. DOLGOPOL, M.D.

(From the Pathologic Laboratories of the Willard Parker Hospital, New York 9, N.Y.)

A new rickettsial disease was recognized in July, 1946, following an outbreak of an epidemic in one of the boroughs of New York City (Shankman¹). This mild febrile disease with an eruption not unlike atypical chickenpox was named "rickettsialpox."

Greenberg and Pellitteri² of the New York City Health Department identified the disease as a separate entity. Huebner, Stamps, and Armstrong³ of the National Institute of Health established the rickettsial etiology of the disease and developed a diagnostic complement-fixation test. Huebner, Jellison, and Pomerantz⁴ traced the vector of the infection, a rodent mite (*Allodermanyssus sanguineus*), and the reservoir, the domestic mouse. The clinical picture was reported by Shankman¹ and by Greenberg, Pellitteri, Klein, and Huebner.⁵

The disease is characterized by fever of about 1 week's duration and a maculo-papulo-vesicular rash. The eruption may be present on any part of the body, including the face, but the palms and soles are rarely affected by the rash. Occasionally the lesions may appear on the oral mucosa. Many patients suffer from photophobia and sore throat. Enlargement of lymph nodes and spleen occurs in some cases.

The majority of patients also have an *initial lesion* which develops at the location of the bite of the mite. It is usually found in some area covered with clothing or with hair (Shankman⁶), but none of the patients has felt the bite. The lesion is a dark red papule from 0.5 to 1.5 cm. in diameter at the base, with a deep-seated vesicle filled with fluid which is clear at first, but later becomes cloudy. The vesicle dries to a black eschar. The initial lesion persists for about 3 weeks. A scar remains after the sequestration of the eschar. Clinical manifestations of the disease begin 1 week after the appearance of the initial lesion.

REPORT OF CASES

Biopsies of skin were made on 6 patients, and a lymph node was excised in one case. Three patients who were admitted to the Willard Parker Hospital during the original investigation of the disease had resided, permanently or temporarily, in the area where the disease was prevalent. Three other patients who became ill several months later lived in other parts of the city, had never been in the locality of the epidemic outbreak, and had had no contact with anyone having a disease with an eruption.

* Received for publication, August 9, 1947.

Case 1

M. K. was a white female, 20 years old. Her mother became ill on July 7, 1946, and recovered 10 days later. The patient noticed a lesion resembling an insect bite on her left shoulder on July 15. On July 22 she had a headache; on July 23, fever, loss of appetite, and pains in the muscles. On July 24 she had shaking chills, and her temperature rose to 104° F. During the next 2 days she developed photophobia, sore throat, and a rash on the neck, body, and extremities, appearing in crops. She entered the Willard Parker Hospital on July 24, volunteering to cooperate in the study of the disease.

Her temperature on admission was 103.2° F.; pulse, 100. The skin was flushed, the conjunctivae were congested. A sparse rash was present on the trunk and extremities. It consisted of irregular erythematous macules gradually rising to a central or eccentric papule surmounted by a minute vesicle sometimes covered with a scab. Enlarged lymph nodes were present in the posterior cervical region and in the right axilla; the spleen was 2 fingersbreadth below the costal margin. A brownish crusted lesion ½ inch in diameter was present on the left shoulder.

Laboratory Examinations. On the day of admission a specimen of blood for animal inoculation was obtained by Dr. Robert J. Huebner of the National Institute of Health. The blood count on July 27 showed 5,000 white blood cells, with 43 per cent neutrophils, which on subsequent examinations rose to 8,400 and 8,250 white blood cells, with 47 and 49 per cent neutrophils. The urine on July 27 was free of albumin, but showed a few hyaline casts; on July 29 it was free of casts. The blood culture taken on July 29 was sterile. The Kline test was negative. Agglutinations with typhoid O and H, paratyphoid A and B, and brucella antigens were negative; with proteus OX 19, positive at 1:80. On July 31 the agglutinations were: typhoid O, positive at 1:20; typhoid H, negative; paratyphoid A and B and brucella, negative; *Past. tularensis*, negative; *B. proteus* OX 19, positive at 1:20. Chemical examination of the blood showed normal findings.

On July 28 (seventh day of illness, fifth day of rash) a skin lesion beneath the right breast and a left inguinal lymph node were removed for microscopic examination (Figs. 9 and 10).

Course in the Hospital. The temperature went down by lysis on July 28. The rash was gone on July 31 leaving a brown pigmentation, the lymph nodes were no longer palpable, the spleen was less firm, but still palpable. The initial lesion became a reddish brown papule. The patient was discharged on July 31.

The specimen of blood inoculated by Dr. Robert J. Huebner³ into mice and guinea-pigs produced illness in mice 9 days later. One mouse was killed and was found to have large abdominal lymph nodes and spleen. Passages from these organs produced a similar disease in other mice and guinea-pigs. Rickettsiae were recovered from the yolk sacs of fertilized eggs cultured with the brain of a mouse from the second passage which had been inoculated intracerebrally. Animals inoculated with these yolk sacs developed the same illness as those inoculated with the patient's blood, and an antigen prepared from the yolk sacs gave a highly specific complement-fixation test with a later specimen of blood of this patient in dilution of 1:512, and positive reactions with sera of other patients in the stage of convalescence. A rising titer was observed where repeated tests had been made.

Case 2

H. B., a white male, 34 years old, noticed a red papule on his right shoulder on July 17, 1946, another lesion in the left axilla on July 23, and became ill on July 25. The rash appeared on July 26. The highest temperature was 103° F. He was admitted to the Willard Parker Hospital on July 27. The rash was of the same character as in the first patient. There was a slight enlargement of cervical lymph nodes and a marked enlargement of axillary nodes. The spleen was barely palpable. Biopsy of the skin was performed on July 28 (Fig. 7). The patient was discharged on August 3, with only a few papules left. The complement-fixation test with the M. K. (case 1) antigen 25 days after the onset of illness was positive at 1:320.

Case 3

R. R., a white female, 50 years old, stayed for 10 days in the home of her daughter where three members of the family were ill. She never noticed an insect bite and had no initial lesion. On the tenth day of her visit, on July 25, she had chills and fever. Between July 27 and 30 she developed a rash, first on the neck, later on the abdomen, with tender inguinal lymph nodes. She was admitted to the Willard Parker Hospital on July 30. On July 31 she had papules surmounted by shiny vesicles on the skin, and a nodule on the hard palate. The temperature was 103° F. A papulovesicular lesion from the abdomen was removed for microscopic examination (Fig. 8). The temperature became normal on August 2. She was discharged on August 7.

Case 4

M. T., a white female, 46 years old, living in a part of the city where no cases of the disease had previously been reported, noticed a slightly itchy "lump" over the right scapula on January 21, 1947. On January 26 she developed a rash on the face and felt sufficiently ill to stay in bed. On January 28 she was seen by a physician who considered the possibility of chickenpox, but on January 30 referred her to the Willard Parker Hospital with a diagnosis of typhus fever.

On admission, on January 30, she had a temperature of 103.4° F.; pulse, 130; scattered macules and papules on the face, trunk, and extremities; and a large, deep red, firm nodule over the right scapula, covered with a dry, scaly epithelium. There was cervical and axillary lymphadenopathy, more marked on the right side. On January 31 the temperature was 100° F. A piece of skin containing the initial lesion and a macule of the rash was removed from the region of the right scapula (Figs. 1, 2, and 3). She was discharged on February 4, with a fading rash. The complement-fixation test for rickettsialpox on January 31 was negative; on March 12, positive at 1:32.

Case 5

L. L. was a Puerto Rican female, 23 years old. She had been in the United States for 3 months, living in a house infested with mice, in a part of the city where the disease had not been reported previously. On January 30, 1947, while bathing, she noticed a small pimple in the right inguinal region. On January 31 she had a headache, pain in the neck, temperature, and an eruption on the face. The following day she had a scattered eruption on the body.

On admission to the Willard Parker Hospital on February 2 she had a temperature of 99.4° F., with papules on the face, chest, abdomen, and extremities, some with vesicles. A small papular lesion, scratched open, was present in the right inguinal region. There was no lymphadenopathy. On February 3 her temperature was normal. A specimen was removed for biopsy from a flat papular lesion, 4 mm. in diameter, in the right deltoid region (Figs. 4, 5, and 6). She was dis-

charged on February 10. The complement-fixation test for rickettsialpox on February 4 was negative; on March 31, positive at 1:32.

Case 6

M. B. was a colored female, 44 years old, who lived in a part of the city where rickettsialpox had not been observed previously. She was admitted to the Willard Parker Hospital on April 24, 1947, with complaints of headache, pain in the left side of the neck, malaise, dizziness, mild chills, and fever of 5 days' duration, and of a mild rash on the face and back which had appeared on the preceding day. The temperature on admission was 100.4° F.; pulse, 84. She had a faint generalized erythema with punctate nonerythematous lesions, and sparse erythematous papules, some with whitish centers, over the abdomen. The pharynx was congested, the tongue coated, and the spleen was 2 fingersbreadth below the costal margin. The right inguinal area was slightly tender, but no lymph nodes were palpable. The liver was palpable. The diagnoses considered on admission were rickettsialpox and typhoid fever. Agglutinations with *Bacterium typhosum*, *Bact. paratyphosum* A and B were negative, with *Bacillus proteus* OX 19, positive at 1:40. On April 25 the patient was afebrile. The spleen was 2 fingersbreadth below the costal margin. A biopsy was taken from a papular lesion. She was discharged on April 29. The complement-fixation test for rickettsialpox on April 28 was positive at 1:8; on May 29, positive at 1:32.

MATERIAL FOR BIOPSY

The material for biopsy was fixed in Regaud's solution for 30 hours, embedded in paraffin, and stained with Giemsa's stain, the technic recommended by the National Institute of Health for the demonstration of the rickettsiae. Additional sections were stained with hematoxylin and eosin.

Initial Lesion

The initial lesion excised from the scapular region of case 4 was a dark red, deep-seated, firm nodule 8 mm. in diameter (Fig. 1). A pustule covered with dry and scaly epithelium occupied the center of the nodule. It measured 5 mm. in diameter and was raised 2 mm. above the base. When the lesion was sectioned, a small amount of yellow fluid escaped and the pustule partly collapsed. The section showed that, while the lesion was deep-seated, the pustule proper was superficial. The corium beneath the pustule was indurated, pale pink, rather moist, and contained grossly visible dilated blood vessels. Microscopically, the pustule was situated entirely within the epidermis. The epithelium was elevated in a vault-like manner; its cells were compressed and showed coagulation necrosis and polymorphonuclear infiltration of the deeper layers. The basal epithelium was present only at the angle of the pustule, and there the intra-epidermal character of the pustule became apparent (Fig. 2). The rest of the basal layer was missing, and the floor of the pustule was formed by the exposed corium. The floor of the pustule was slightly and superficially infiltrated with polymorphonuclear cells at the center of the lesion but showed no necrosis

of the stroma. The pustule was filled with serum, shreds of necrotic epidermis, and polymorphonuclear leukocytes. The capillaries in the deeper layers of the corium were dilated, and some contained polymorphonuclear cells. The endothelium of other capillaries was swollen, bulging or desquamating into the lumen, and occasionally showed mitotic figures. Several smaller capillaries contained pale pink homogeneous material partly occluding the lumen (Fig. 3). Mononuclear cells and occasional polymorphonuclear cells were scattered in the corium and were more densely aggregated near the blood vessels. The mononuclear cells were lymphocytes, mast cells, and peculiar cells with a small amount of dark or light cytoplasm, one or two processes, a large, bulging nucleus, and a dark nucleolus; connective tissue cells and fibroblasts were present also. The mast cells were most numerous at the periphery of the lesion, especially in mononuclear infiltrates surrounding the hair roots and other cutaneous appendages. They were elongated or polyhedral, were densely packed with metachromatic material, and appeared as solid, dark purple masses under low and high-dry magnifications; under the oil-immersion lens, however, the metachromatic granules often could be seen within the body of the cell, and always in its processes. The cells with a small amount of cytoplasm and a bulging nucleus were found to be similar to the lymphoid wandering cell illustrated in Maximow and Bloom's textbook.⁷ No plasma cells were seen in the infiltrates.

Rash

Four maculopapular and two papulovesicular lesions were examined. In a maculopapular lesion the epidermis was intact or was slightly thinner than in the adjacent skin, with some scaling on the surface (Fig. 4). In the upper layer of the corium there was an increased number of connective tissue cells. Capillaries, veins, and cutaneous appendages (hair roots, sebaceous and sweat glands, and also arrectores pilorum muscles and nerve trunks) were surrounded by dense collections of cells, but there was no diffuse infiltration of the corium (Figs. 4 to 7). Few cells with vesicular nuclei were present. The mast cells were more numerous than in the initial lesion (from 5 to 15 in a high-power field). In some cases numerous pyknotic nuclei and nuclear fragments were scattered among the cells of the infiltrates. Lymphocytes were numerous, but polymorphonuclear cells were rare; in 2 cases they were eosinophilic. Homogeneous pale pink thrombi or strands of fibrin were seen in some capillaries, and the endothelium of many capillaries bulged toward the lumen. In one case occasional mast cells were found beneath the desquamating endothelium of small veins.

The epithelium of the sweat glands was sometimes hyperchromatic, but showed no desquamation; occasional mast cells invaded the walls of the sweat glands.

In a fresh papulovesicular lesion the vesicle occupied the entire thickness of the epidermis. The greater part of the epithelium forming the thick top of the vesicle showed vacuolization and some disintegration of the cells with fragmentation of the nuclei, but the basal layer was largely intact (Fig. 8). There was no "ballooning" or hyalinization of the epithelial cells as seen in chickenpox. The vesicle contained a small amount of fibrin and a few polymorphonuclear cells. The upper layer of the corium was streaked with elongated nuclei of leukocytes migrating toward the vesicle and contained a thrombosed blood vessel. Perivascular and some diffuse mononuclear infiltrations were present in the deeper layers of the corium beneath the vesicle, but on either side of that area the picture was the same as in maculopapular lesions.

In the healing papulovesicular lesion the vesicle was located in the cornified layer of the epidermis and contained only thin protein material (Fig. 10). The epithelium beneath the vesicle had been restored, but was slightly concave. Periadnexal infiltrates and vascular changes still were present in the corium.

Lymph Node

The lymph node excised from the inguinal region of case 1 was bean-shaped, moderately soft, and measured 8 by 5 mm. The cut section was pale pink and slightly granular. Microscopically, the lymphoid tissue showed nothing remarkable except a few eosinophils here and there and several groups of mast cells. The stroma of the hilus was edematous and contained several small groups of mast cells and scattered cells with large vesicular nuclei similar to those seen in the infiltrates in the corium of the initial lesion (Fig. 9).

DISCUSSION AND SUMMARY

The gross appearance of the initial lesion of rickettsialpox is similar to that of the primary lesion of scrub typhus and of the tache noir of Marseille fever.

No microscopic description of the tache noir seems to be available in the literature, but the microscopic picture of the initial lesion of rickettsialpox closely resembles the histologic findings in the primary lesions of scrub typhus as described by Allen and Spitz.⁸ The pustules in these two diseases are identical. The difference in the inflammation of the corium is largely quantitative. The area of polymorphonuclear infiltration of the corium in rickettsialpox is more limited

and more superficial than in scrub typhus, and there is no degeneration of the connective tissue. The vascular changes also are less severe. The cellular infiltrates follow the same perivascular and periadnexal character as in scrub typhus, but the plasma cells are absent and the mast cells are more numerous.

The maculopapular rash of rickettsialpox is microscopically similar to the eruptions of other rickettsial diseases in regard to the character and distribution of the cellular infiltrates, but the infiltrates are much heavier than in any other rickettsial disease. Karyorrhexis may be quite prominent, and mast cells densely packed with metachromatic granules are numerous. Plasma cells are absent. The vascular changes in rickettsialpox closely resemble those of scrub typhus, but are less severe. Incomplete homogeneous thrombi and the absence of arteritis and hemorrhages in the eruption of both diseases distinguish them from typhus, Rocky Mountain spotted fever, and Marseille fever.

The vesicle of the rash is unique in a rickettsial disease. The epithelium at the top of the vesicle shows vacuolization and some disintegration of the cells with karyorrhexis. The basal epithelium is largely intact. The corium immediately beneath the vesicle shows some migration of polymorphonuclear cells and a slight diffuse mononuclear infiltration, but in the papular portion of the eruption which forms the base of the vesicle the changes in the corium are the same as in other papular lesions.

The lymph node showed no necrotic changes characteristic of scrub typhus, but the presence of mast cells in the lymphoid tissue and in the stroma of the hilus was an evidence of some damage to the node.

Although rickettsiae (*R. acari*) had been recovered from the blood of some patients, they have not been found in sections of the skin lesions or of the lymph node.

Rickettsialpox is a mild disease with a uniformly good prognosis, but in the early stage the clinical picture may be confusing, especially in the absence of an initial lesion. The diagnoses of chickenpox, typhus fever, and typhoid fever were considered in some of our patients at the onset of illness.

Several laboratory procedures have been devised as diagnostic aids, but some of them proved to be impractical. Cultivation of the rickettsiae from the blood of the patients is so complicated and protracted as to make it completely unsuitable for diagnostic purposes. The complement-fixation test is the best diagnostic laboratory procedure, but it usually requires at least two specimens of blood taken 3 or 4 weeks apart, if the first test is negative. Microscopic examination of the rash has its place as an aid in the diagnosis of rickettsialpox, especially if

a suspicious disease appears in an area previously free from that disease. The histologic examination of a skin lesion may be completed long before the report on the second specimen of blood is available and will help in securing earlier confirmation of the diagnosis.

I wish to thank Drs. Arthur C. Allen and Sophie Spitz for making their material on rickettsial diseases available for study and for helpful suggestions in the preparation of this paper.

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DESCRIPTION OF PLATES

PLATE 24

FIG. 1. Case 4. Initial lesion of rickettsialpox, at least 10 days old. The bisected specimen at the right shows the superficial collapsed pustule and the indurated, slightly congested area in the corium.

FIG. 2. Case 4. Initial lesion of rickettsialpox. Angle of the pustule showing the intra-epidermal character of the pustule and loss of the basal epithelium a short distance from the angle. The epithelium of the wall is compressed, and polymorphonuclear cells from the lumen of the pustule invade the necrotic inner layer of the epithelium. Giemsa's stain. $\times 220$.

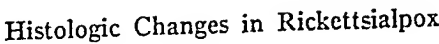


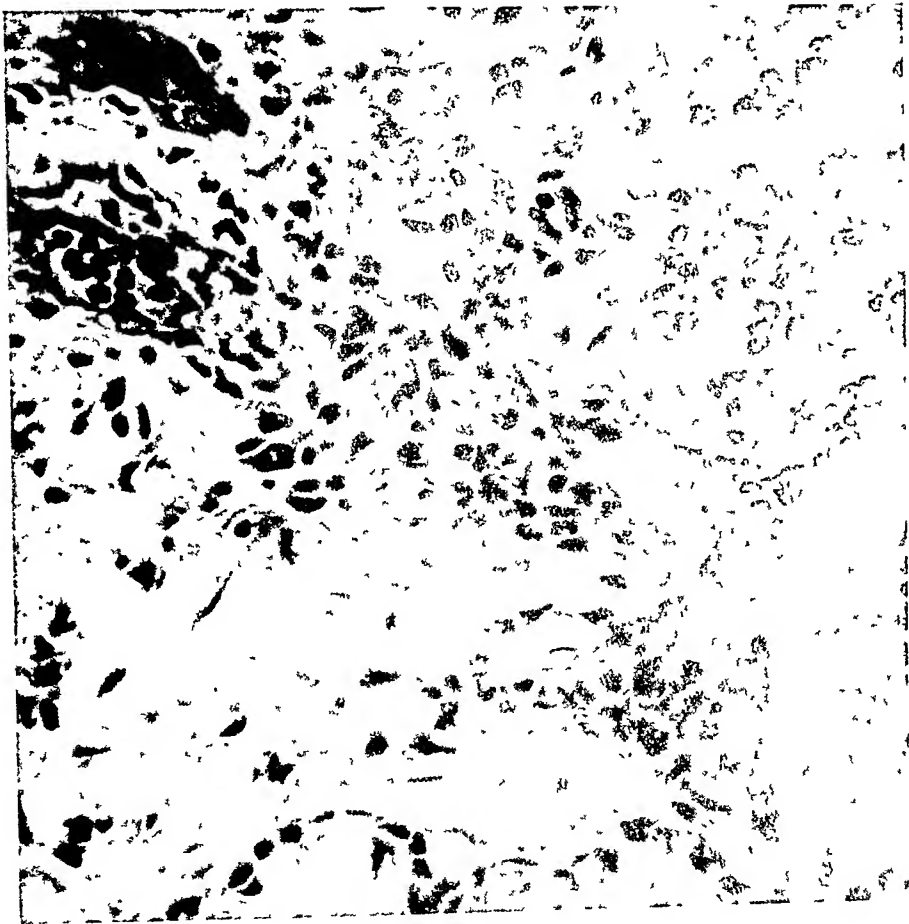
PLATE 25

FIG. 3. Case 4. Cellular infiltration and congestion of the corium beneath the pustule. Swollen endothelium in the branching capillary; mitotic figure in an endothelial cell in the right lower corner of the field. Homogeneous material and desquamated endothelium in the lumen of the capillary in the upper left corner. Cells with vesicular nuclei adjacent to the capillary; several similar cells in the central portion of the field. Giemsa's stain. $\times 440$.

FIG. 4. Case 5. Papule of rickettsialpox. Slight scaling of the epidermis. Perivascular and periadnexal cellular infiltrates. Mast cells appear as large dark bodies. Giemsa's stain. $\times 100$.

FIG. 5. Case 5. Detail from Figure 4. Homogeneous material partly fills the lumen of a capillary. Nuclear fragments and mast cells are present in the perivascular infiltrate. Mast cells are present also near the arrector pili muscle. Giemsa's stain. $\times 660$.

3



4



5



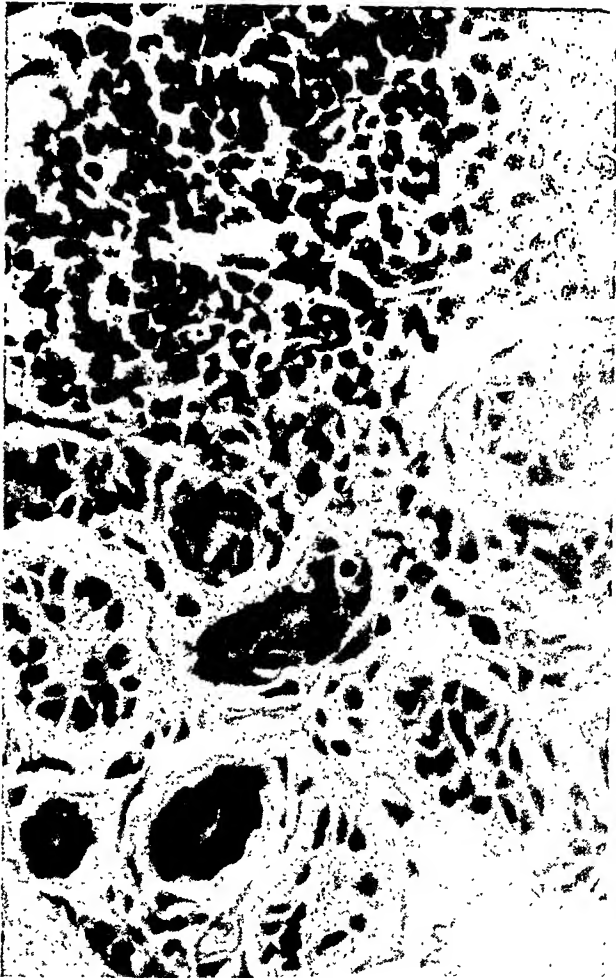
Dolgopol

Histologic Changes in Rickettsialpox

PLATE 26

- FIG. 6. Case 5. Cellular infiltrate around a sweat gland in another area of the corium beneath the same papule seen in Figures 4 and 5. Mast cells and nuclear fragments are scattered among the lymphocytes. Giemsa's stain. \times 440.
- FIG. 7. Case 2. Maculopapular rash of rickettsialpox. Mast cells in the infiltrate around a sebaceous gland and arrector pili muscle. Giemsa's stain. \times 440.
- FIG. 8. Case 3. Fresh papulovesicular rash of rickettsialpox. Intra-epidermal vesicle covered with a thick layer of epithelium showing hydropic changes and some disintegration of the cells. The basal layer is largely preserved. Some nuclei of migrating polymorphonuclear cells are present in the upper layer of the corium. A slight diffuse mononuclear infiltration is seen in the lower layers of the corium. Hematoxylin and eosin stain. \times 100.

6



7



8



Dolgopol

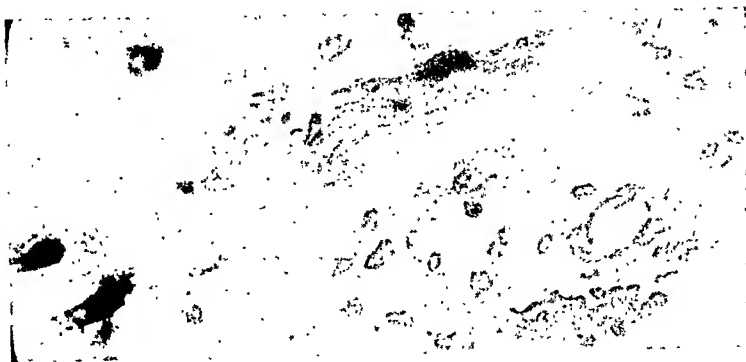
Histologic Changes in Rickettsialpox

PLATE 27

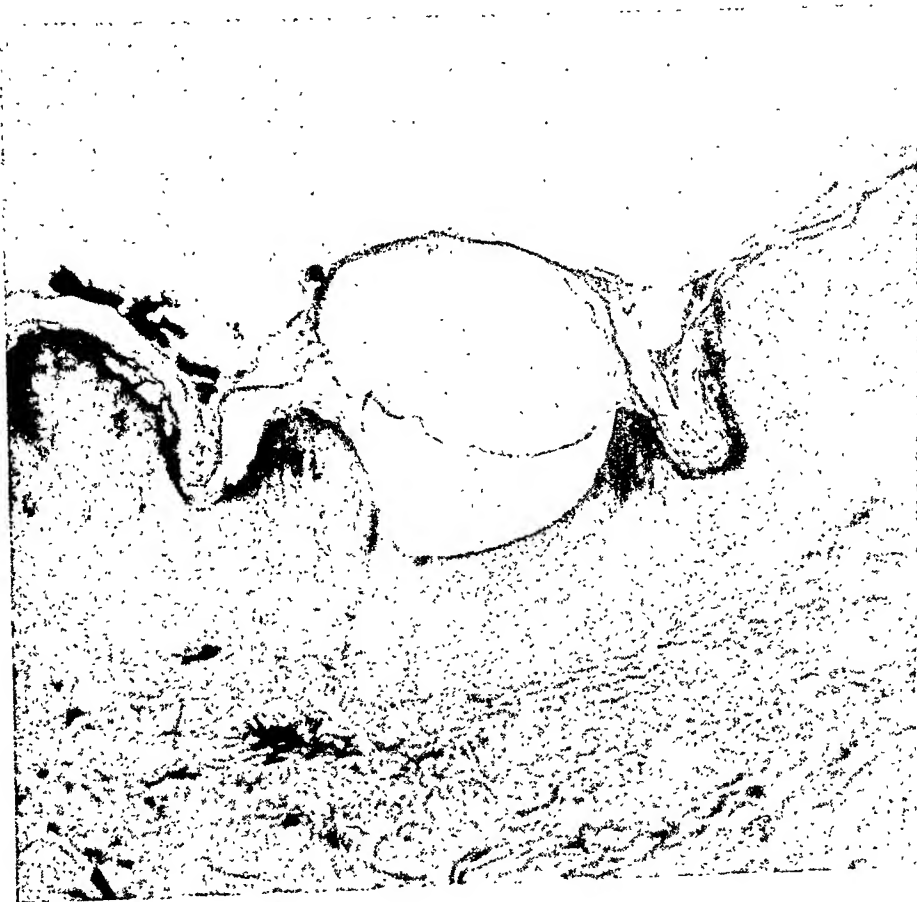
FIG. 9. Case 1. Mast cells in the stroma of the hilus of an inguinal lymph node. Giemsa's stain. $\times 440$.

FIG. 10. Case 1. Drying vesicle of a papulovesicular rash of rickettsialpox. The vesicle is located in the cornified layer of the epidermis. The epithelium at the base of the vesicle is restored to normal thickness. Giemsa's stain. $\times 100$.

9



10



Dolgopol

Histologic Changes in Rickettsialpox

STUDIES ON AMEBOID MOTION AND SECRETION OF
MOTOR END-PLATES

X. EFFECTS OF SLOW NERVOUS ACTION OF DISUSE ON THE STRUCTURE
OF NERVE ENDINGS, NEUROSOMES, AND MUSCLE FIBERS *

EBEN J. CAREY, M.D.,† EUGENE HAUSHALTER, LEO C. MASSOPUST, FRANK
GAROFALO, JOHN LYNCH, DENIS TABAT, and ELI SOCOLOFF

(From the Department of Anatomy, Marquette University School of Medicine,
Milwaukee 3, Wis.)

The morphologic effects of inactivity upon the nerve endings of the neuromuscular apparatus in skeletal muscle have remained an unexplored field. The surgeon empirically has discovered and rediscovered the beneficial effects of early ambulation after an operation. The structural changes, however, in nerve and muscle, produced by immobilization are practically unknown. The knowledge of the pathologic changes induced by disuse in nerve endings may give a clear insight into the normal nervous discharge and the substantial dependence of nerve and muscle.

The experimental study herein reported is a product of the hypothesis that normally there is a discharge of fine neurogenic granules into muscle.¹ Considerable evidence has been presented that under physiologic conditions there is a tenuous and evanescent secretion of very fine neurogenic granules into muscle and that these granules undergo rapid periodic discharge, diffusion, and disappearance by hydrolysis. It is assumed that under the pathologic conditions of disuse, by various methods, the neurogenic secretion accumulates slowly and forms enlarged droplets. These enlarged neurogenic droplets are considered to be the products of retardation in the rate of the periodic discharge, diffusion, and dissolution by hydrolysis. During the early period following the disuse of muscle, a pathologic dissociation may be produced of the neurogenic and myogenic substances that normally constitute muscle.

The object of this paper, therefore, is to present conclusive morphologic evidence of the pathologic changes produced in nerve, nerve ending, neurosome, and muscle by disuse, in comparison with their normal structure associated with normal use.

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MATERIALS AND METHODS

Under aseptic surgical technic, the Achilles tendon of the right gastrocnemius muscle was completely severed by transverse section from the calcaneus, and 3 mm. of the distal end of the tendon were excised in 300 white rats (*Mus norvegicus*). The gastrocnemius muscle of the left leg was allowed to remain intact and used as a control of the effects of normal use. At 24-hour intervals the morphologic changes in the right gastrocnemius muscle were compared with the normally used muscle of the left side in each of 10 rats over a 30-day period. Segments of the control and the experimental gastrocnemius muscles and sciatic nerves were simultaneously subjected to the same histologic technics. Of 60 additional white rats, 4 were selected at 48-hour intervals following tenotomy. A ligature tied at the cut end of the muscle had at its free end suspended weights that varied from 10 to 30 gm. depending upon the size of the muscle. The living muscle *in situ* was gradually restretched, prior to excision for gold impregnation, for periods of 10 to 15 minutes, alternating with 5 minutes of rest, for 3 to 5 hours.

The right hind legs of 60 other rats were completely encased in plaster of Paris casts from the toes to and including the hip joints. The cast of the hind leg was continuous with a partial body cast for stability and anchorage. The left hind leg was free for walking. Four of these rats were selected at 48-hour intervals after the application of the cast. The structural changes in the right immobilized gastrocnemius muscle were compared with those in the normally used left muscle. Comparison was made likewise with the changes at the same periods of time following disuse by tenotomy and by the cast.

The method of gold impregnation and teasing of whole muscle fibers, previously described,¹ was found superior to any other neurologic technic for the detection of the structural changes of the nerve endings in muscle. This method was checked against the current popular ones in which silver or methylene blue is used. The muscles were likewise stained with osmic acid, sudan III, sudan black, and scharlach R, as well as with ordinary stains such as hematoxylin and eosin, after fixation with formalin, Zenker's fluid, or other fixatives.

The variable structure of the motor end-plates produced by gold (Figs. 1 and 2) and methylene blue (Figs. 3 and 4) in teased fibers of the same gastrocnemius muscle of the pseudo-chameleon (*Anolis carolinensis*) was contrasted with that produced by silver (Figs. 5, 6, and 7) in longitudinal frozen sections after formalin fixation. The chameleon was used in this experimental study because the nerve endings are two to four times larger than those found in any mammalian muscle

and the various structures may be readily compared. These three neurologic technics produced the same changes in mammalian nerve terminals as they did in those of the chameleon muscle. The revelation of the true microscopic structure of the nerve ending, therefore, is dependent upon the histologic technic employed. The granules of the sole plate of Kühne are more clearly demonstrated by the gold method than by either methylene blue or silver.

The nerve terminals are constricted by the silver method. The neurofibrillae and nuclei are more clearly revealed by this method than by any other. The gold method, followed by teasing of the whole muscle fiber, was, however, superior to any other neurologic technic for the demonstration of the normal and pathologic discharge of neurogenic substances from nerve endings into muscle. When there was a copious discharge of neurogenic substances from nerve endings that had a large diameter demonstrated by the gold method, similar portions of the same muscle subjected to the silver method revealed clear-cut neurofibrillae in the terminal axons and a periterminal network of Boeke between these axons and the anisotropic substance of the muscle. This periterminal network, therefore, was not a constant finding in all nerve endings. The silver method revealed loops, rings, terminal brushes, and terminal networks in a variable manner in the nerve endings. These were related to the variable structure of the nerve endings demonstrated by the gold technic. The conclusive evidence, therefore, that the motor nerve endings were microscopic glands of internal secretion was better demonstrated by the gold method than by any other neurologic technic.

The left and right sciatic nerves were stained with gold, silver, methylene blue, osmic acid, and various other dyes, to demonstrate myelin degeneration. Nerves and muscles were prepared in whole units and in longitudinal and cross sections. The great distortion, shrinkage, and chemical changes produced by the formalin fixation and alcoholic dehydration used in the silver method were eliminated by the use of the gold method in the study of nerve endings in muscle. The gold chloride reacted directly with the neurogenic axonic substances in nerve and muscle without need of the prior use of formalin fixation.

Formalin produced chemical changes and altered critical features of the structure of the neuromuscular apparatus. No alcoholic dehydrating agent was used in the gold method of teased whole mounts. There was some swelling of the nerve and muscle when they were placed in the citric acid or in lemon juice prior to gold impregnation, but this tended to reveal more clearly than otherwise the real nature of the union of nerve and muscle. It was realized, however, that any artificial

technic did not demonstrate, absolutely, the reality of the living structural union of nerve and muscle. The acid, however, may have acted by stabilizing the products of neurogenic secretion of nerve and muscle before they were either altered or destroyed by the chemical changes produced by the ordinary fixatives used in histologic study.

The teasing of whole muscle fibers was a better technic than cutting muscle into sections for observation of the whole neuromuscular apparatus and the anatomic relationships and changes of the epilemmal axon, hypolemmal axon, granules of Kühne, cross striations, and the granular and agranular muscle fibers. The failure, in the past, to detect morphologic changes in the nerve endings has been due to the popular choice of certain histologic methods which profoundly alter the structure of the union of nerve and muscle. In the living, this union is transparent, and up to the present time it has defied clear-cut observation, but this problem is still under investigation. The following experimental observations will be confined to the structure of nerve endings of the gastrocnemius muscle in the white rat revealed by the gold technic.

RESULTS

Effects of Normal Use on the Motor End-Plates in Dark and Light Voluntary Muscle Fibers

The normal muscle observed after gold impregnation in teased whole muscle fibers had relatively narrow, dark, and granular muscle fibers (Fig. 8) scattered among others of various diameters which were light and relatively agranular. The granular fibers had coarse, medium-sized, and fine granules. The muscle fibers were classified as hyperchrysophilous, orthochrysophilous, hypochrysophilous, and achrysophilous based upon the intensity of the reaction of the granules with gold. There were multiple gradations of affinity of the granules with gold between the extremes. The nerve endings were usually expanded (Fig. 8) and had a decreased affinity for gold in the light fibers; the endings were usually retracted in the dark fibers.

The granules of Kühne usually formed a dense rim around the retracted nerve endings, whereas there was a quantitative diminution of these granules, to the point of complete depletion, around the extended branches of the expanded nerve endings produced by neuroprotoplasmic streaming. The light halo-like space found between the terminal axons and the granules of Kühne (Figs. 1, 2, and 30) in some normal nerve endings was not a constant finding in all motor end-plates. The evidence suggested that it may represent the interval in which submicroscopic neurogenic particles were discharged and quickly became visible by enlargement through interaction with the colloidal

granules of the muscle substance. This halo-like space is not an artifact but was absent when there was massive transmission of the irritable neurogenic substance into the sarcoplasm of the muscle fiber (Figs. 39 to 62). In a differential count of 5,000 normal nerve endings, the retracted endings varied in length from 20 to 40 μ and the expanded endings from 40 to 60 μ .

The normal muscle in cross section (Figs. 13, 14, and 15) had fibers with coarse, medium-sized, and fine granules in the narrow and medium-sized muscle fibers. The extremely wide fibers had the gold-impregnated material arranged in a reticulum, or there was a marked diminution of the substance with an affinity for gold in the light, agranular fibers. There were variations, however, in the size of the granules in fibers of different diameters. The axon, nerve endings, granules of Kühne, and granules in the muscle fiber all had the same reaction to gold. The size and distribution of the granules were assumed to be related to the different degrees in the process of hydrolysis after the neurosomes were discharged into the muscle.

Effect of Tenotomy on the Dark, Granular Type of Muscle Fiber

During the first 2 to 3 days following tenotomy (Fig. 9), there was frequently found in some muscles a quantitative increase in the frequency of distribution of the dark muscle fibers. Between the 3rd day (Fig. 9) and the 30th day (Figs. 12 and 19) there was a gradual loss of the dark muscle fibers following tenotomy. There was, likewise, a great depletion (Fig. 12) of the nerve supply as well as of the substance of the muscle fiber by the 30th day. In some places, the hypolemmal axons of the nerve endings were absent (Fig. 12) and all that remained 30 to 50 days after tenotomy were clumps of sole plate nuclei. The structural expression of the dark, coarsely granular muscle fiber (Figs. 8, 9, and 13) was determined, therefore, by the presence of the attached and normally functioning muscle with its active innervation. It required a considerable period of time, following disuse by tenotomy, to deplete the muscle fiber of the neurogenic substance normally discharged into the muscle. Whether or not this neurogenic substance discharged into muscle may go through a certain number of cycles of breakdown and resynthesis before it is completely used up, is still undetermined by this experimental study. This neurogenic substance with a variable affinity for gold is found in the sarcoplasm. When it forms a well defined network (Figs. 20, 21, and 22), it corresponds to the sarcoplasmic boundaries of the so-called areas of Cohnheim observed in ordinary histologic preparations after formalin and Zenker's fixation.

From the 2nd to the 15th day (Figs. 25 to 27) following tenotomy the intramuscular capillaries and small veins became clearly visible due to the passive hyperemia associated with disuse.

Effects of Tenotomy on the Discharge of Pathologic Neurosomes from Motor End-Plates

The nerve endings became uniformly and deeply impregnated with gold, and fusiform in shape, between the 4th and 20th days following tenotomy in the rat. There was individual variation in different animals as well as in different fibers of the gastrocnemius muscle in the same animal in the appearance of these morphologic changes (Figs. 23 to 27). The molding of the nerve endings into various shapes, such as oval and fusiform, was produced by the progressive shrinkage of the muscle fiber due to the loss of muscle substance (Figs. 23 to 27, and 40 to 62). The course of the transformation of the motor end-plates into either giant globular or giant fusiform neurosomes may be traced by arranging in sequence a selection from a large number of motor end-plates studied under the microscope (Figs. 40 to 62). The evidence is conclusive that a large mass of material found in the giant neurosomes is contributed by the motor end-plates even though there may be an interaction of this neurogenic material with sarcoplasmic granules in the muscle substance.

During the early period following tenotomy (Figs. 40 to 46), the motor end-plates were uniformly impregnated with gold with little or no evidence of distinct hypolemmal axons. This interaction with gold was due to the presence in the nerve ending of an increased quantity of material with an affinity for gold. The normal and abnormal experimental muscles were run through the various stages of the technic simultaneously. Frequent comparisons of the normal reaction to gold (Figs. 30 to 32, and 35 to 38) were made with the abnormal reactions of the motor end-plates (Figs. 28, 29, 33, 34, 39, and 40 to 62). The pathologic changes following tenotomy were proved to be due, therefore, not to a faulty technic or to overimpregnation with gold, but to the intrinsic changes produced in the neuromuscular apparatus following tenotomy.

On the 10th to the 15th days following tenotomy (Figs. 10 and 11) there was a discharge of either small or large gold-impregnated neurosomes from the hyperchrysophilous motor end-plates. In some places the epilemmal axons manifested a decrease in the quantity of material with an affinity for gold (Figs. 10 and 11) in comparison with both the normal innervation and the innervation 48 hours following tenotomy (Figs. 8 and 9). There was structural manifestation of various stages in the genesis and discharge of giant globular and fusiform

neurosomes from the oblong and spindle-shaped nerve endings which were hyperchrysophilous (Figs. 10 and 11, and 40 to 62).

From the 3rd to the 15th days following tenotomy, in certain places in the muscle a progressive increase in the number of giant neurosomes was found. From the 15th to the 30th days following tenotomy, there was a progressive decrease in their number. The discharged giant neurosomes were irregularly scattered in the myoplasm. Some of them were uniformly and deeply impregnated with gold. Some were light in the center and dark at their tapering ends. Others were light at the ends and dark in the center. Still others were very faintly impregnated with gold, and their contained granules were arranged in cross striations undergoing progressive alignment with the cross striations of the muscle fiber.

These fusiform neurosomes varied from 10 to 275 μ in length and from 4 to 60 μ through the widest transverse diameter. In some places there were small, oblong and fusiform neurosomes, similar in structure and staining reaction to gold to those observed during the early stages after nerve section in denervated muscle.¹ The large fusiform neurosomes were produced by a streamlining effect (Figs. 63 to 75) of the cross-striated myoplasm during the process of migration of the neurosomes from the nerve ending and dispersion into the protoplasm of the muscle fiber. Some of these neurosomes in transverse sections were immediately under the sarcolemma whereas others occupied the center (Figs. 16 to 18, and 20 to 22) of the muscle fibers. During disuse atrophy there was a progressive loss of the normal dark muscle fibers and of the normal coarse granules (Figs. 16 to 18, and 20 to 22) of neurosomes. The granules were either very fine and dispersed, or aggregated into the giant fusiform neurosomes. In cross sections (Figs. 16 to 18, and 20 to 22) the giant neurosomes were either uniformly deeply impregnated with gold, or they had a light center, or a rim of granules faintly reacting to gold.

This morphologic evidence strongly suggested that the visualization by photography of the pathologic nerve impulse was determined by the accumulation of neurogenic substances produced by a retardation in the rate of discharge, diffusion, and dissolution by hydrolysis of the neurogenic secretion from the motor nerve ending. This neurogenic secretion interacted with the muscle substance. The friction of the myogenic fluid slowed down the transmission of the giant neurosomes. The resisting drag of the viscous myoplasm on the migrating neurosomes was associated with the formation of whirlpools or eddies. The resulting eddies or vortices were detected by the turbulence of the related cross striations at the prow and in the wake of the giant

neurosomes. The streamlining of the neurosomes resulted in tapering ends. The pointed rear end is more important in streamlining than the front end. In some locations (Fig. 65) the neurosomes assumed the "teardrop design" of streamlining with one end blunt and convex like that of a torpedo. This design was modified, however, by the fact that the neurosomes are not inert particles moving in the muscle protoplasm but are subjected to colloidal chemical action and reaction with the muscle substance. Progressive changes in the hydrolysis of the giant neurosomes were clearly evident (Figs. 63 to 75).

Effects of Disuse from Casts on the Production of Pathologic Neurosomes

The effects produced on the nerve endings of the gastrocnemius muscle by immobilizing in a plaster cast the right hind leg of a rat were essentially similar to those found after disuse by tenotomy. The deformations of the nerve endings after disuse produced by the casts occurred later than those demonstrated after tenotomy. This may be associated with the slower rate of muscle atrophy after immobilization by a cast than that produced following tenotomy. The neurosomes discharged from nerve endings during the early stages after the application of the cast were usually more nearly spherical than those generated by tenotomy (Figs. 47 to 56).

The neurosomes in tenotomized muscle were generally elongated and fusiform (Figs. 57 to 75). It is assumed that the configuration of the neurosomes was determined by the rate of atrophy and the physical changes of viscosity of the myoplasm produced by disuse, as well as by a slowing down in the rate of secretion, diffusion, and hydrolytic dissolution of the neurosomes.

Effects of Tenotomy on the Nerve Supply of the Gastrocnemius Muscle

During the first 10 days after the right gastrocnemius muscle was tenotomized there was an increase in the weight of the nerve fibers supplying the muscle. Corresponding lengths of 5 mm. of the control left and experimental right nerves, from their entrances into the muscles, were excised and weighed in 30 rats. There was an increase in the weight of the nerve fibers of the right experimental side that varied from 10 to 40 per cent over that of the left control nerve fibers. This increase was due to: (1) edema associated with the passive hyperemia of the intraneural blood vessels; (2) increase in diameter of the axis cylinder; and (3) accumulation of lipoidal material due to demyelination of the medullated nerves. These pathologic products of nerve dystrophy following disuse augmented the size of the axis cylinder

(Figs. 28, 29, 33, and 34) and were then discharged periodically in a centrifugal direction into the dystrophic muscle (Figs. 39 to 75).

In frozen sections after formalin fixation and silver impregnation the enlarged epilemmal and hypolemmal axons during the first week after either tenotomy or the application of a cast showed an increased number of neurofibrillae. This increase was associated with a dilatation and increase in quantity of neurogenic material in the axon when studied with gold (Figs. 28, 29, 33, 34, and 39 to 46). There was an apparent increase in number of the nuclei in the sole of some nerve endings observed by the silver method following atrophy of disuse. This may have been an aggregation by migration of neighboring nuclei in the muscle and not an absolute increase in total number of nuclei in the whole muscle fiber.

Effect of Restretching the Tenotomized Muscle

The re-establishment of partial muscle stretch in some fibers of the muscle by the regenerative attachment of the tendon to the subcutaneous tissue is a variable which we took into consideration in evaluating our results. In some of the rats in our series, the tendon of the muscle was freshly cut at 7-day intervals to eliminate the effects of the partial re-establishment of stretch by union of tendon to subcutaneous tissue. The experimental evidence strongly suggested that the loss of normal muscle stretch determined the retardation in the rate of discharge, diffusion, and disappearance by hydrolysis of the giant neurosomes. This conception was supported by the great decrease in the number, and in some muscles the total absence, of the giant neurosomes in the series of 60 living muscles experimentally restretched *in situ* before excision and gold impregnation. Four muscles were restretched at 48-hour intervals until the 30th day after tenotomy. The living muscles were restretched for periods of 10 to 15 minutes alternating with 5 minutes of rest, for 3 to 5 hours. The muscles were restretched by a ligature tied at the distal cut end of the muscle, from which weights were suspended that varied from 10 to 30 gm. depending upon the size of the muscle.

Effect of Tenotomy on Muscle Weight

The gastrocnemius muscles lost 30 to 50 per cent of their weight, compared with the normal, during the first month after tenotomy. There were individual variations in the rate of atrophy and in the morphologic changes of the neuromuscular apparatus and fibers of the gastrocnemius muscles. These variations were found not only in muscles from different animals examined after the same time interval following tenotomy, but also in different fibers in the same muscle. It

was necessary, therefore, to survey great numbers of muscle fibers to detect the average trend of the morphologic changes.

DISCUSSION

The disuse of muscle by tenotomy and from a cast produces dystrophy or a nutritional disorder of nerve and muscle. Langley² recognized the analogy between denervated and fatigued muscle. Others have noted the resemblance between the atrophy of muscle due to loss of nerve supply and that due to extreme starvation.

What is the underlying mechanism of muscle atrophy? The answer to this question involves our conception of the structure of muscle tissue. Langley,³ upon the basis of chemical experiments, identified two constituents: (1) the irritable substance in the sarcoplasm, and (2) the contractile molecule in the myofibrillae. Tower⁴ has defended, likewise, the proposition that sarcoplasm and myofibrils are discrete. The possibility that these constituents of the muscle cytoplasm form a continuum has been considered also by Tower. Hürthle and Wachholder,⁵ however, concluded that the sarcoplasm is merely a schematic abstraction of the histologic structure of muscle.

Langley³ mentioned two possibilities regarding the localization of special excitable substances in muscle cells. He found that curare decreases the excitability of the muscle to the nicotine stimulus and assumed that it decreases also the irritability of the muscle to stimuli arriving by the nerves. He assumed that the compounds which these poisons form with the muscle are less irritable and conductive than the normal muscle substance. Since neither curare nor nicotine, even in large doses, prevents direct stimulation of muscle from causing contraction, it is obvious that the muscle substance which combines with nicotine or curare is not identical with the substance which contracts. Langley called this specially excitable constituent the receptive substance. It receives the stimulus and, by transmitting it, causes contraction. He stated that if nicotine causes contraction of the fibrillae and not of the sarcoplasm, there are two possibilities: the receptive substance may be part of the sarcoplasm, or it may be a radical of the contractile molecule. Langley clearly stated that his hypothesis of a specific excitable substance in muscle required that the nerve impulse should not pass from nerve to muscle by an electric discharge, but by the secretion of a special substance at the end of the nerve, a theory suggested in the first instance by du Bois-Reymond.⁶ This theory was placed on a substantial basis by Loewi,⁷ Dale, Feldberg, and Vogt,⁸ and others. Langley considered the problem from a theoretical point of view, regarding the localization of the special receptive substance as either in the immediate neighborhood of the nerve ending or dissemi-

nated throughout the length of the muscle fiber. He concluded from his experiments that it is the receptive substance in the muscle cell, rather than the nerve endings, which is stimulated or paralyzed by curare and nicotine. The receptive substance was also involved in some change during fatigue, according to Langley's conception.

Denervation atrophy abolishes the morphologic differences between the types of muscle fibers. Knoll and Hauer⁹ observed that the thick fibers decreased in size much faster than the thin ones and that after 35 days there was no longer any distinction among the fiber types in the atrophied muscle. The granules which appeared when the normal thin muscle fibers were excised and observed fresh, disappeared after denervation. The appearance of these granules in the normally thin fibers, therefore, was associated in some way with the normally functioning innervation.

The atrophy of disuse of muscle following tenotomy or the application of a cast likewise abolishes the morphologic differences between the types of muscle fibers. There was a rapid diminution in size of the thick fibers and a progressive loss of dispersion of the characteristic dark gold-impregnated granules in the thin muscle fibers. During the early period following tenotomy of innervated muscle there is a massive aggregation of the granules into coagulated clumps that vary in size. Disuse slows down the rate of the nerve impulse and forms large viscous droplets discharged and delayed in dissolution from the nerve ending. The pathologic neurogenic substance associated with the abnormal nerve impulse retarded in rate of discharge from the dystrophic nerve ending is morphologically detected by this method of retardation. Inactivity abolishes, therefore, the normal agitation of dispersion of the neurogenic granules and reticulum in intimate relationship with the myofibrillae.

This clumping of neurogenic substances in the sarcoplasm, by disuse of the muscle, may be considered an intramuscular method of denervation of the myofibrillae inside of the muscle fiber. The efficiency of normal innervation appeared to depend upon the normal activity of diffusion of neurogenic granules that undergo progressive decrease in size by hydrolysis and thereby make intimate contact with the colloidal myofibrillae. The electric signs of the normal nerve impulse are assumed to be associated with the substantial transfer of the tenuous and evanescent neurogenic granules which excite the myofibrils to the rapid type of contraction and which likewise underlie the normal tonus of muscle.

The basic mechanism in the physiologic processes of use, increased use by exercise, and disuse, resulting, respectively, in muscle maintenance of health, in hypertrophy, and in atrophy of disuse and disease, is

unknown. Young¹⁰ stated that "the theories of the causes and nature of muscular atrophy are numerous but none is conclusive." This conclusion is held likewise by Carlson and Johnson.¹¹ The parts of the living body increase and decrease in size in proportion to the functional demand or use, but the essential underlying cause of these changes has remained elusive. The changes in physiologic structure during use and disuse of the neuromuscular apparatus have remained an unexplored field in spite of our present information that loss of motor innervation results in the following characteristic changes: (1) the quantitative decrease of the substance in the muscle fiber, and (2) the hyperexcitability of the skeletal fibers to mechanical and chemical (acetylcholine) stimuli. The hyperexcitability manifest by fibrillations may be prevented by quinidine, but Solandt and Magladerer¹² observed that the denervated muscle continues to shrink. The slow, incoordinate activity of fibrillation, therefore, appears not to be the cause of muscular atrophy. It has been demonstrated by Gutmann and Gutmann,¹³ Hines,¹⁴ Eccles,¹⁵ and Solandt, DeLury, and Hunter¹⁶ that the volume of denervated muscle may be fairly well maintained for a certain period by appropriate electrical exercises. Evidently, periodic optimum tension of traction and contraction (work) of muscles anchored to their attachments is necessary for muscle maintenance during health. Suggestive evidence was demonstrated previously that an optimum periodic tension of differential growth is necessary for the genesis of both smooth and skeletal muscle.¹⁷ Evidence was presented also that anatomic and experimental dampers to the lateral expansion of the stretched muscle fibers produce a replacement of muscle by fibrous tissue.¹⁸

The normal tension, or stretch, and work of the intact muscle attached to origin and insertion appear to be necessary for the normal rate of discharge of granules from the motor end-plates. Abundant evidence has been produced which appears to support the statement that under normal conditions there is a cloud of very fine, evanescent granules discharged rhythmically into the muscle from the motor nerve endings. This secretion is periodic and under normal conditions the rate of diffusion and disappearance of the granules by hydrolysis is very rapid. Evidence has been presented which suggests that there is a close relationship between the granular and agranular muscle fibers and the rapid cycle of discharge, diffusion, and disappearance of the neurogenic granules in muscle. When the rate of discharge and hydrolysis of the neurogenic secretion has been retarded by disuse, there is a correlated change in the morphologic expression of the discharge from the motor nerve endings.

This secretory process from the nerve endings may be slowed down by disuse of the innervated gastrocnemius muscle following tenotomy. By tenotomy, the normal stretch or tension of the muscle is destroyed. The lax muscle fibers released from one attachment are analogous to the broken strings of a violin. The detached strings are incapable of normal vibratory response because of loss of tone or tune. The rate of discharge of neurogenic substance into the muscle appears to depend upon the reciprocal interaction between nerve and muscle. The normal mechanical tension of the attached muscle fibers appears to determine the normal periodical flow of neurogenic substances into the muscle. The normally attached muscle fiber appears to act like an alternate pressure and suction chamber upon the nerve ending. This nerve ending appears to be a biologic jet valve or ejector of the neurogenic secretion. Under normal conditions the rate of discharge, diffusion, and disappearance is excessively rapid. Under conditions of disuse this rate is greatly retarded. There is a characteristic structural expression reflected by the atrophy of disuse of the neuromuscular apparatus following tenotomy. The release of normal muscle stretch by tenotomy decreases the demand for, and therefore the rate of, neurogenic discharge into the abnormally flaccid muscle fibers.

This laxity of the muscle fibers slows down the normal rate and permits accumulation of the discharge of the neurogenic secretion. The longitudinally striated myoplasm is composed of (1) the myofibrils which vary in width and in distinctness, and (2) the sarcoplasm containing the neurogenic substance which, likewise, varies in size from large, pathologic neurosomes to the very small, physiologic neurosomes. Some of the muscle fibers are agranular. The granular and agranular muscle fibers may, therefore, be designated, respectively, as the neurosomic and aneurosomic fibers. These granules are large in the narrow, dark, granular muscle fiber. The so-called sarcoplasm contains these neurosomes.

The normally discharged neurosomes react in a variable manner to gold, thionin, and lipoidal stains. Evidence now at hand supports the statement that the problems of fatty metamorphosis, Zenker's hyaline degeneration, and the pathogenesis of dystrophy of muscle are closely related to aberrations of the neuromuscular apparatus and alterations in the secretion of neurosomes into muscle. In the past, many of the fine physiologic neurosomes have been identified variously as the interstitial granules of Kölliker,¹⁹ the J and Q granules of Holmgren,²⁰ and the nutritional liposomes of Albrecht,²¹ Bell,²² and Denny-Brown.²³

In some of the light, agranular fibers there is a fine network of neurogenic material in the sarcoplasm, and in its meshes are found the cross

sections of single or multiple myofibrils. This neurogenic network, in some places, surrounds each myofibril, whereas in other locations in the fibers of the same muscle this fine network is absent. When irregular groups of myofibrils are surrounded by the coarse network of neurogenic material, the so-called areas of Cohnheim are formed in the cross section of the muscle fiber. There is a high degree of variability in the presence and absence of areas of Cohnheim. These Cohnheim areas are not constant, fixed, structural arrangements of the myofibrils in each muscle fiber, as is now assumed. The problem of muscle structure requires a complete restudy based on experimental, controlled conditions.

The evidence presented in this paper supports the principle of the *double dependence* of nerve and muscle proposed by Young.¹⁰ He stated that muscle receives its stimulation from nerve and exercises constraint against other muscles or outside forces. Muscle will atrophy if given too little direction from above, as after total denervation or isolation of lower from upper neurones; it will also atrophy if it is left relaxed and, therefore, cannot contract against resistance. However, until the structure of nerve and muscle is more adequately understood than it is at the present time, the problem of muscle maintenance, hypertrophy, and atrophy will remain unsolved. The study of the after-effects of infantile paralysis and other primary and secondary diseases and dystrophies of nerve and muscle will not be clarified until the real structure of nerve and muscle under physiologic and pathologic conditions is known.

The experimental evidence presented in this paper substantiates the proposition that one important factor underlying the mechanism of muscle atrophy of disuse is the substantial loss of the normal neurosomes discharged from nerve to muscle. The loss or decrease in the amount of this normal morphologic transmitter associated with the electric signs of the normal nerve impulse parallels the decrease in the mass of the myoplasm following inactivity of muscle.

SUMMARY

Experimental evidence has been presented which supports the following statements:

1. The apparent structure of the nerve ending in the skeletal muscle fiber is dependent upon the specific histologic technic employed. The gold method, followed by the teasing of whole muscle fibers, is better than other technics employing silver or methylene blue for demonstrating the normal and pathologic neurosomes discharged from the motor nerve ending into the muscle fiber.

2. The visualization by photography of the morphologic transmis-

sion of an increased mass of substance from nerve to muscle, associated with the pathologic nerve impulses, was determined by a slowing down of the rate of nervous action by inactivity of the associated muscle.

3. During the early stages of disuse atrophy of rat muscle produced by tenotomy or by the application of a cast, the neurogenic secretion is retarded in the rate of discharge, diffusion, and dissolution by hydrolysis. The enlarged droplets of nerve substance in muscle may be observed in different stages of solution and photographed for detailed study. The normal rate of discharge, diffusion, and dissolution is so rapid that detailed microscopic study of the physiologic neurosomes is difficult.

4. There is a gradual loss of the dark, granular muscle fibers during the first month after tenotomy, as well as a rapid decrease in mass of the light, thick muscle fibers. The physiologic production of the granular fibers appears to be dependent upon the normal function of the innervation which discharges normal neurosomes into the muscle fiber. The evidence suggests that the coarse, medium-sized, or fine granules and the gold-impregnated network in the sarcoplasm are different stages in the hydrolysis, diffusion, and intimate commingling of the irritable nerve substance in the colloidal and fibrillar muscle substance. The inconstant structure of the areas of Cohnheim appears to correspond with the phase of the gold-impregnated network formed by the progressive hydrolysis of the neurosomes in the sarcoplasm.

5. The pliability of the active muscle appears to depend upon the normal colloidal compounding of the nerve and muscle substances. The muscle stiffness associated with disuse is accompanied by an abnormal aggregation of the nerve substance into large clumps inside the muscle fiber, resulting in a loss of the normal dispersion of fine granules of nerve substance. This abnormal clumping of nerve substance in the inactive muscle fiber appears to be a form of partial intramuscular denervation. The normal dispersion of fine neurogenic particles in muscle is dependent upon the normal reciprocal activity of nerve and muscle. Inactivity slows down the periodic neurogenic discharge of granules into muscle and appears to increase the viscosity and delay the disappearance of the pathologic nerve fluid flowing into muscle.

6. The giant fusiform neurosomes that appear during the early period following tenotomy disappear or are greatly reduced in number when the living muscle *in situ* is restretched adequately prior to excision and gold impregnation.

7. The increase in size and weight of the dystrophic nerve fibers supplying the gastrocnemius muscle is associated with the following structural changes: (1) edema and passive hyperemia of the intra-

neural blood vessels; (2) increase in diameter of the axons; (3) progressive demyelination, the products of which are discharged centrifugally through the axon into the dystrophic muscle. The evidence appears to support the conclusion that there is a continuous generation of pathologic material by the dystrophic nerve for 2 or 3 weeks following tenotomy. Over a period of time, the mass of abnormal nerve substance discharged into the dystrophic muscle would be considerably greater than the initial mass of nerve fibers at the time of tenotomy.

8. One factor in the atrophy of disuse of muscle appears, therefore, to be a substantial loss of the discharge of the normally fine neurosomes from nerve endings into skeletal muscle. This loss of physiologic neurosomes is associated with decrease in the mass of the myoplasm.

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DESCRIPTION OF PLATES

The photomicrographs of Figures 1 to 75 are from either teased whole muscle fibers (gastrocnemius muscle) and nerve endings, or from cross or longitudinal sections of muscle from the white rat (*Mus norvegicus*) and pseudo-chameleon (*Anolis carolinensis*). The muscle was previously prepared by one of three methods, namely, gold, methylene blue, or silver. The photographs were direct contact prints from negatives exposed through the microscope and subsequently enlarged. They may be compared directly, therefore, with those previously published. In the plates, "epa" designates the epilemmal axon; "hya," the hypolemmal axon; "NS" or "Gns," small or giant pathologic fusiform neurosomes or neurogenic particles discharged from nerve endings into the myoplasm; "Kg," Kühne's granules, or normal physiologic neurosomes; "NE," nerve endings. The negatives and prints were not retouched.

PLATE 28

FIGS. 1 to 7. Differential structural effects of three different neurologic techniques are demonstrated on the neuromuscular apparatus in the same gastrocnemius muscle of the pseudo-chameleon (*Anolis carolinensis*), used because the motor end-plates are two to four times larger than in mammalian muscle. The variable appearances of the motor end-plates produced by gold (Figs. 1 and 2) and methylene blue (Figs. 3 and 4) in teased whole muscle fibers are contrasted with those produced by silver (Figs. 5, 6, and 7) in longitudinal sections after formalin fixation. The differences in the structure of the nerve terminals produced by these three techniques are found in mammalian as well as chameleon muscle. The granules of Kühne (Kg) are clearly visible in some of the preparations made with gold impregnation (Figs. 1 and 2) but are invisible or poorly shown in those made with methylene blue (Figs. 3 and 4) and silver (Figs. 5, 6, and 7). The tremendous deformation of the nerve terminals produced by formalin fixation, silver impregnation, and alcoholic dehydration (Figs. 5, 6, and 7) is clearly apparent. No such deformation occurs when the gold (Figs. 1 and 2) or the methylene blue (Figs. 3 and 4) methods, which require no fixative, are employed. The nuclei contain nucleoli and are in close relation to the terminal filaments of the nerve endings. These nuclei are more clearly observed after silver impregnation (Figs. 5, 6, and 7). Rings, loops, terminal brushes, and networks are found on certain nerve endings after the silver method is used. The nuclei form clear oval spaces surrounded by granules after the use of gold impregnation. The technic of choice, therefore, to demonstrate the neurogenic granular discharge (Kg) into muscle from the nerve endings is gold impregnation followed by teasing of the whole muscle fibers (Figs. 1 and 2). The periterminal network of Boeke is found in silver preparations, but in place of this network a copious accumulation of the granules of Kühne is demonstrated by the gold method. When these granules are depleted or absent around the nerve terminals, the periterminal network is absent. This evidence suggests that the preparation of muscle by the silver method converts the granules of Kühne into a network. This network is found between the end of the nerve and the anisotropic substance of the muscle fiber. $\times 850$.

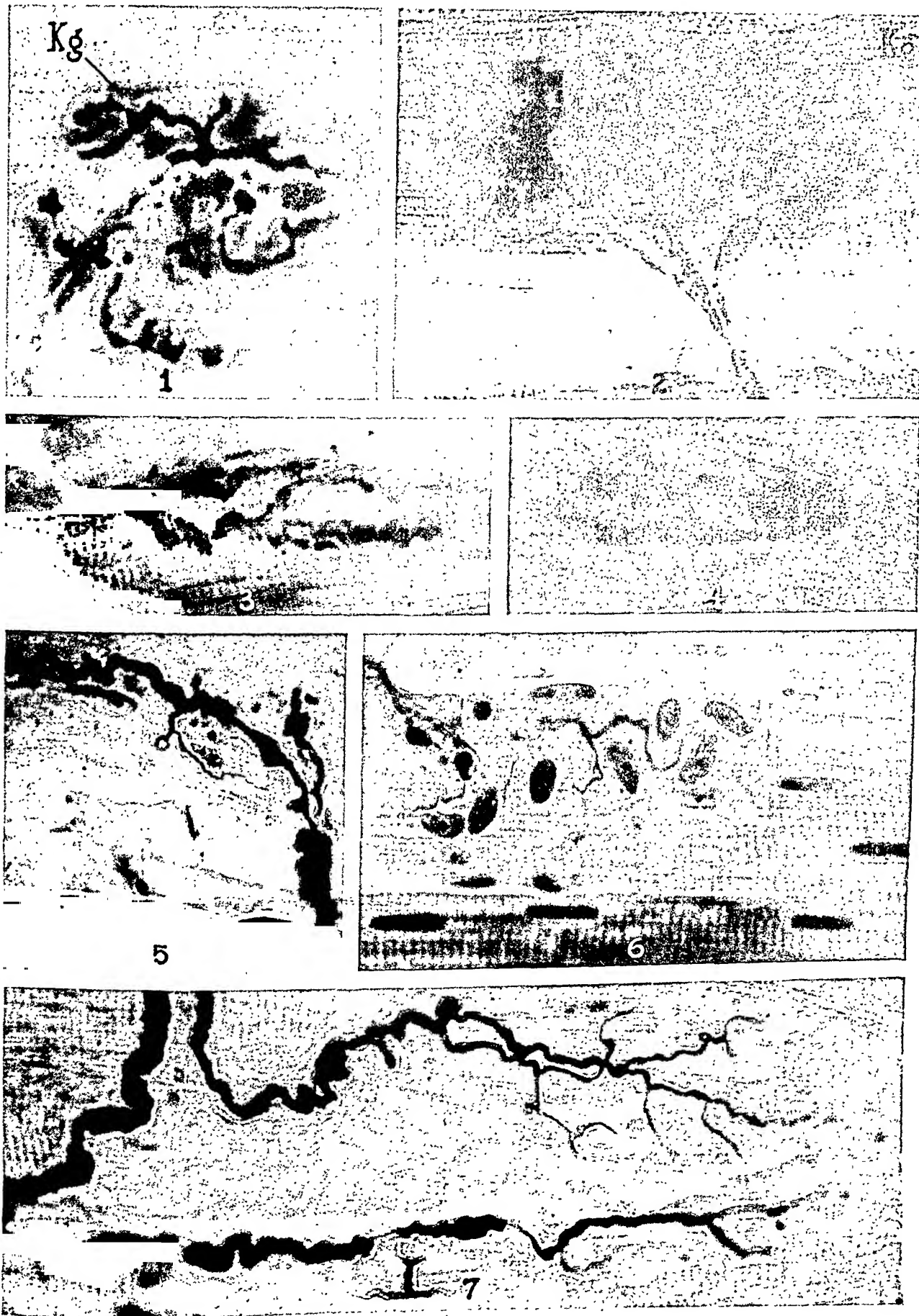


PLATE 29

Figs. 8 to 12. Gold technic, teased whole muscle fiber. $\times 200$.

- FIG. 8. Spray of medullated nerve fibers and nerve endings from a relatively normal gastrocnemius muscle removed from an etherized rat. The retracted nerve ending is in a dark, coarsely granular muscle fiber, and the expanded nerve endings are in the light, finely granular or agranular muscle fibers. The normally functioning muscle manifests fractional contraction, the structural expression of which is the differential structure of the muscle fibers and nerve endings. The dark, granular muscle fiber is related to a hyperchrysophilous nerve ending surrounded by fine granules of Kühne or physiologic neurosomes. The light, relatively agranular fibers are related to expanded nerve endings, which are in various stages of depletion of the surrounding physiologic neurosomes. The types of muscle fibers are related to the different phases in the discharge, diffusion, and dissolution by hydrolysis of normal neurosomes secreted from the nerve endings, as well as to the granules secreted by the nuclei in the sole plate and in the muscle.
- FIG. 9. Spray of medullated nerve fibers and nerve endings in the gastrocnemius muscle 48 hours after tenotomy. There is an increase in number of the dark, granular muscle fibers, and in the diameter and staining capacity of the epilemmal axons.
- FIG. 10. Motor innervation of the gastrocnemius muscle 15 days after tenotomy. The dark, granular muscle fibers are decreased in number. There is hyperchrysoiphilia of the fusiform nerve endings. There is retardation in the rate of discharge, diffusion, and dissolution by hydrolysis of the neurogenic granules, manifested by a substantial accumulation of the droplets into giant fusiform neurosomes.
- FIG. 11. Motor innervation of gastrocnemius muscle 10 days after tenotomy. The dark, granular muscle fibers are decreased in number. There is hyperchrysoiphilia of the nerve endings and a slowing down in rate of discharge, diffusion, and dissolution, manifested by small pathologic neurosomes in the muscle. The small or giant neurosomes may appear at any time from 1 to 21 days after tenotomy.
- FIG. 12. Depletion of innervation and gradual loss of dark muscle fibers in the atrophic gastrocnemius muscle 30 days after tenotomy. The dark, granular muscle fiber, therefore, is the structural expression of the normally functioning motor nerve supply that periodically discharges physiologic neurosomes into the muscle fiber.

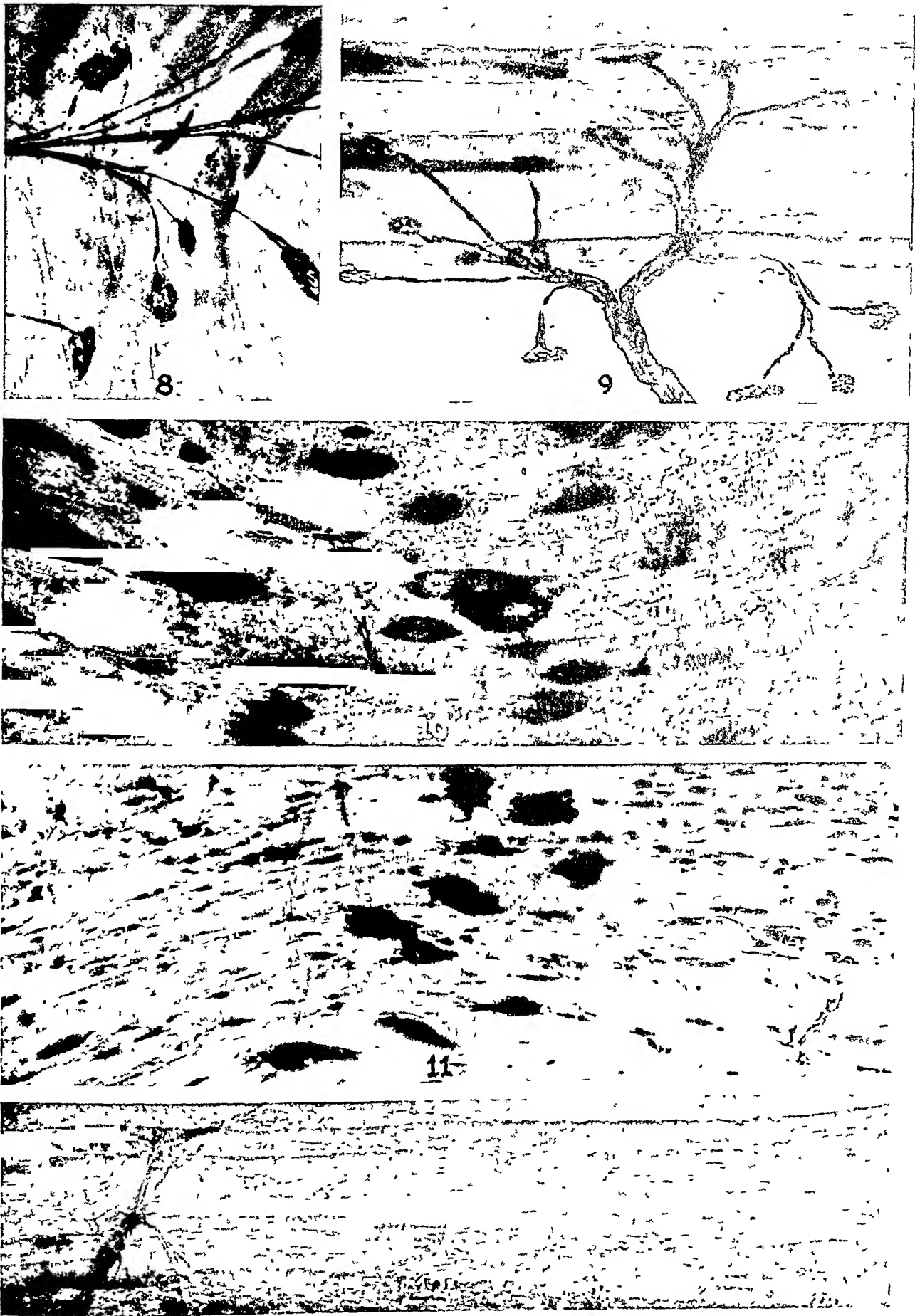


PLATE 30

Figs. 13 to 19. Gold technic, frozen cross sections. $\times 400$.

FIGS. 13, 14, and 15. Cross sections of the normal gastrocnemius muscle fibers of the rat are demonstrated. Relatively narrow, coarsely granular fibers, medium-sized and finely granular fibers, as well as wide, relatively agranular and reticulated fibers are evident. The network is clearly presented in some of the fibers (Figs. 20 to 22). This fine, chrysophilous network of the large muscle fibers, and the dissolution of this net, appear to be stages in the hydrolysis of the large granular neurosomes found in the small, dark muscle fiber. These multiform neurosomes occupy the space of the sarcoplasm found between the myofibrils and groups of myofibrils.

FIGS. 16 and 17. Cross sections of giant fusiform neurosomes (Gns), in various locations of the gastrocnemius muscle fibers 10 days after tenotomy. These pathologic neurosomes have the same affinity for gold as have the nerve endings (Fig. 17, NE). These neurosomes are either uniformly and densely impregnated with gold or they have a light center composed of fine granules and forming a circle or oval in cross section. The material of these fusiform spaces is soluble in xylol and alcohol, and in ordinary histologic preparations clear clefts in the muscle fiber are produced. The cleavage of the myoplasm described in muscle dystrophy may be produced by these neurosomes which are dissolved in ordinary preparations.

FIG. 18. Cross sections of muscle fibers 15 days after tenotomy. The small pathologic neurosomes are densely stained in a manner similar to the epilemmal and hypolemmal axons of the nerve endings. These pathologic neurosomes are at the periphery, the center, or between these two locations. There are single, double, or multiple neurosomes.

FIG. 19. There is definite atrophy of the muscle 30 days after tenotomy. The dark, granular muscle fiber (Fig. 13) is practically eliminated after 30 days of disuse atrophy.

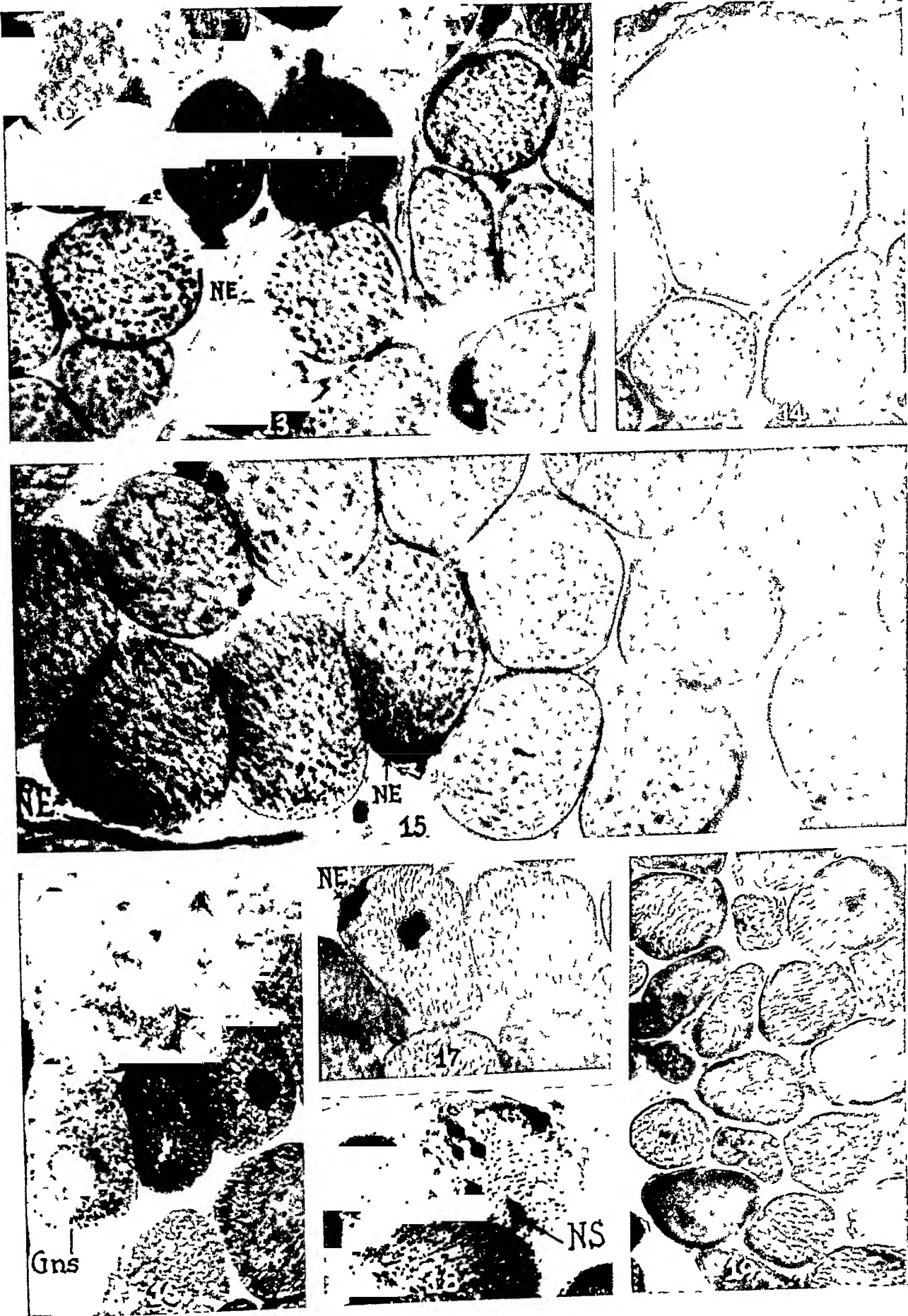


PLATE 31

Figs. 20 to 22. Gold technic, frozen cross sections. $\times 400$.

FIGS. 20 and 21. Cross sections of giant fusiform neurosomes (Gns) in gastrocnemius muscle 10 days after tenotomy. In some muscle fibers the neurosomes form a large dark hub, whereas in other fibers these bodies are found at the periphery under the sarcolemma. In certain fibers (Fig. 21) the agglutination of the gold-impregnated material is found in the small, light muscle fibers undergoing considerable atrophy. In such muscle fibers there appears to be a lack of the normal diffusion of the gold-impregnated material throughout the muscle fiber. The areas of Cohnheim appear to be formed by the coarser strands forming a network of the gold-impregnated material, which has not become accumulated into the giant fusiform neurosomes.

FIG. 22. Cross sections of small neurosomes 15 days after tenotomy of the gastrocnemius muscle. In those muscle fibers in which the gold-impregnated material forms a uniform network, there is an absence of the pathologic neurosomes. Various stages in the aggregation of gold-impregnated granules are found in different muscle fibers. During atrophy of disuse by tenotomy, there is a progressive loss of the characteristic coarse and darkly granular muscle fiber observed in the normal muscle in relationship to a normal innervation (for comparison with Fig. 13).

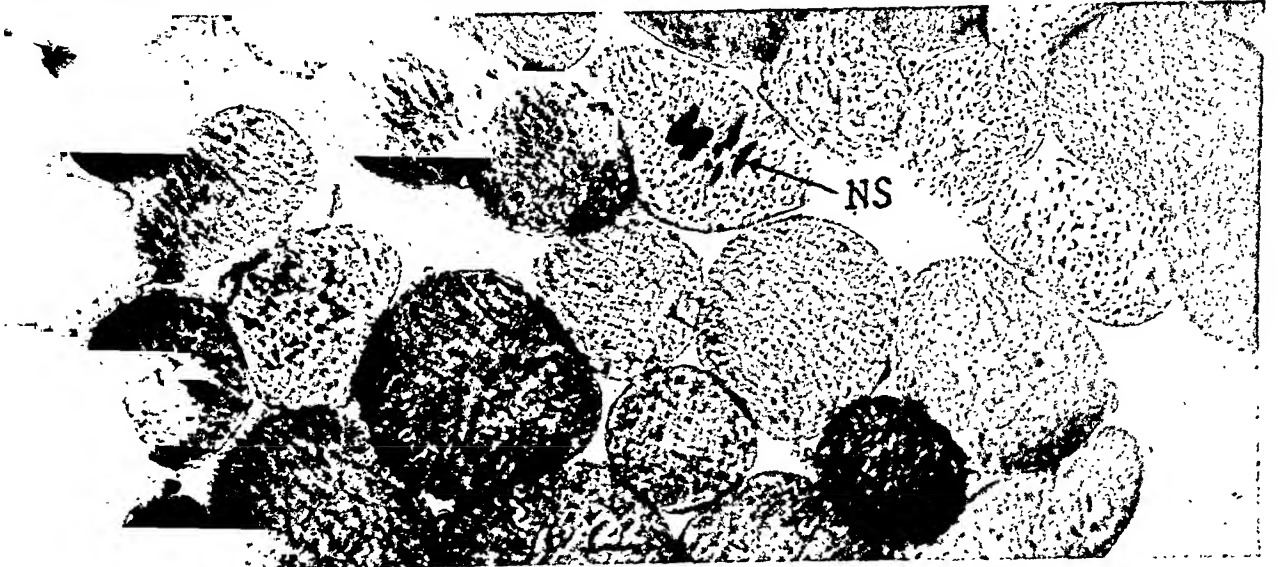
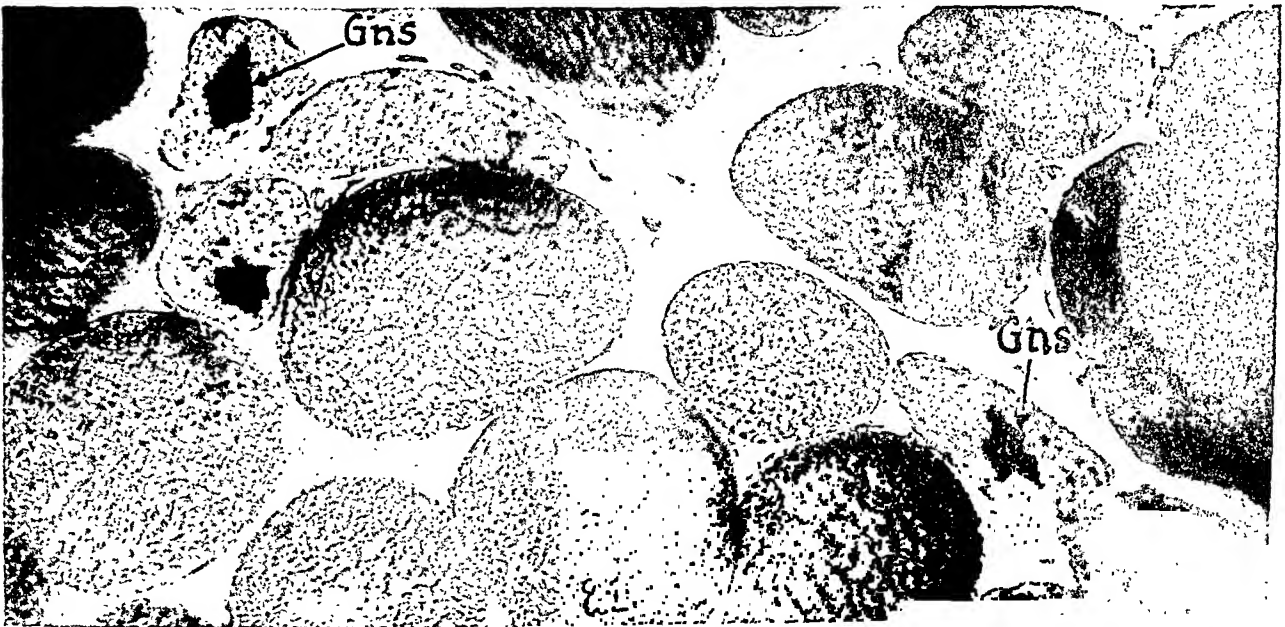


PLATE 32

FIGS. 23 and 24. Innervation of gastrocnemius muscle of the rat 24 hours (Fig. 23) and 96 hours (Fig. 24) after tenotomy. There is a copious discharge of giant fusiform neurosomes (Gns) 96 hours after tenotomy (Fig. 24), as well as increased diameter and staining capacity of the epilemmal axons. The normal epilemmal axons (Fig. 8), and those at 24 hours (Fig. 23) after tenotomy, vary from 1 to 7 μ in diameter, whereas at 96 hours following tenotomy the axons vary in diameter from 1 to 20 μ . Disuse of the innervation following tenotomy produces an enlargement of the axons of the nerve supply because of the delay in, or block to, the neurogenic discharge by disuse, through loss of normal stretch of the muscle fiber. Gold technic, teased whole muscle fibers. $\times 200$.

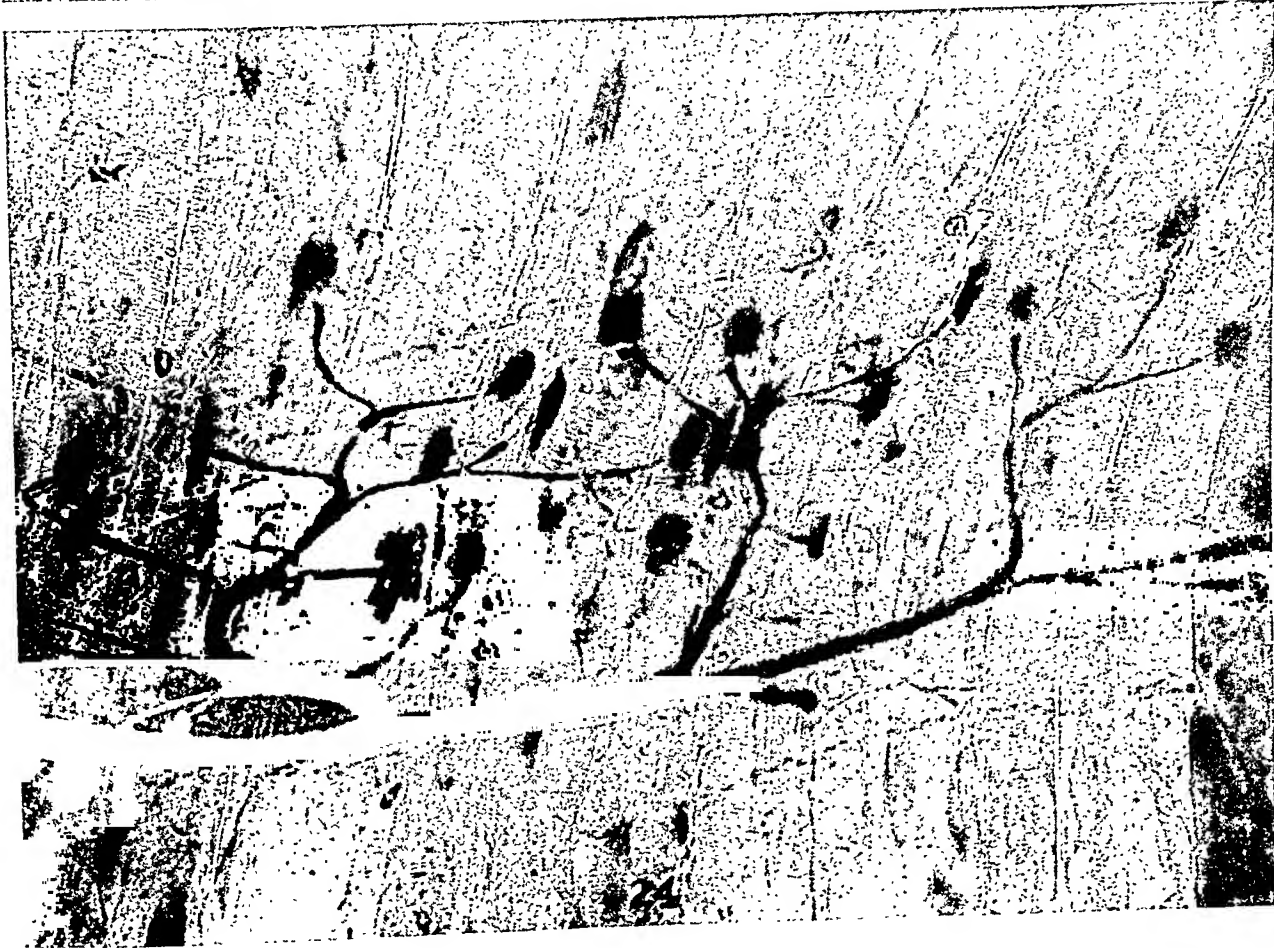
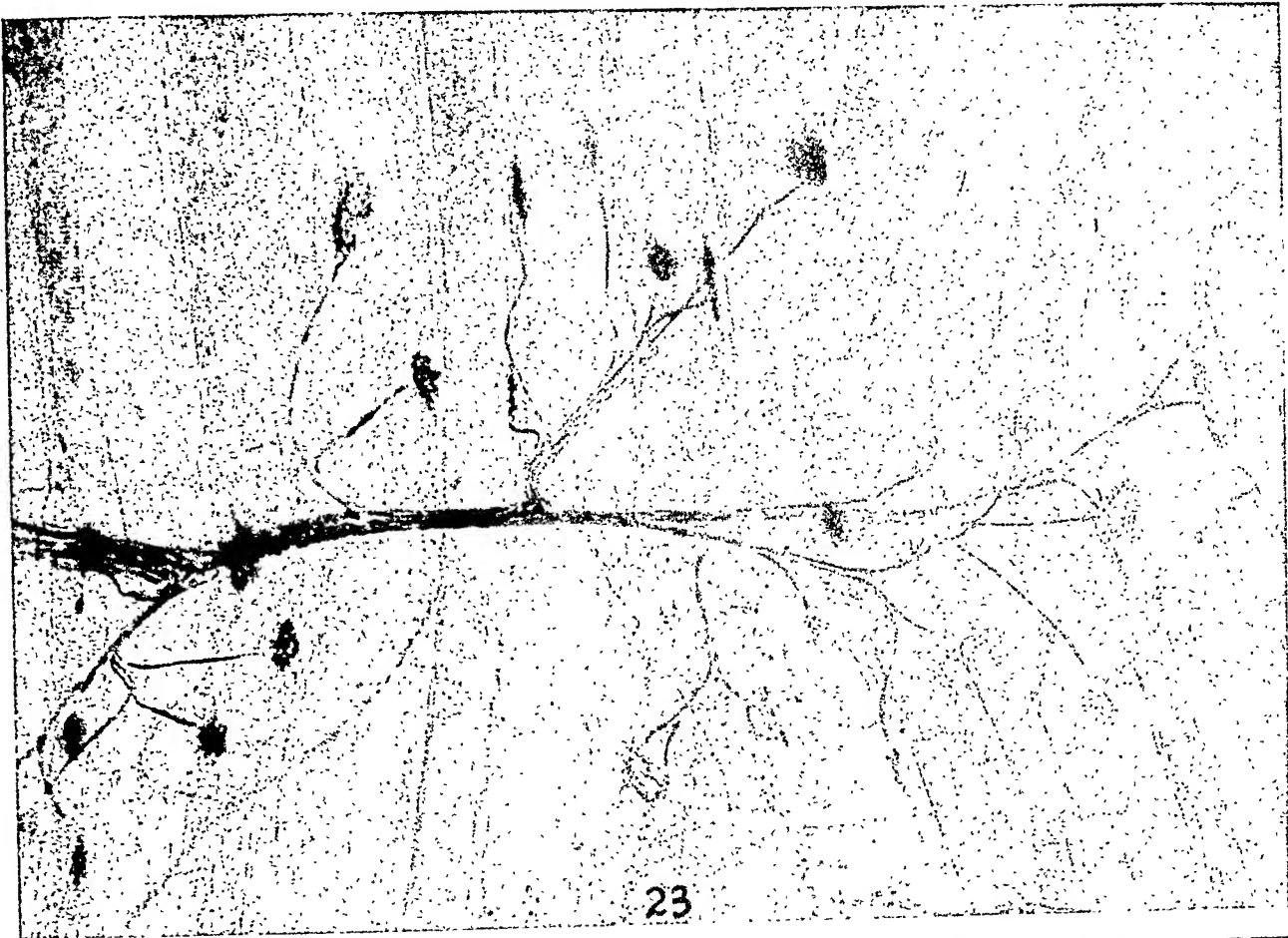


PLATE 33

FIGS. 25, 26, and 27. Innervation of the gastrocnemius muscle of the rat 15 days following tenotomy. The discharge, diffusion, and disappearance by hydrolysis of the giant fusiform neurosomes are greatly slowed by disuse and loss of normal stretch of the muscle fiber. Some of the pathologic neurosomes are hyperchrysophilic whereas others undergoing dissolution by hydrolysis are either hypochrysophilous or achrysophilous. The abnormal structural expression of the neurogenic transmitter substance is the result of retardation in the rate of the chemical influence of nerve on muscle. The structure of the pathologic chemical transmitter is more easily demonstrated than normally by slowing the rate of nerve impulses by disuse following tenotomy. The abnormal transmitter substance accumulates in quantity under slow motion and assumes, by streamlining, the shape of a torpedo that slowly diffuses and dissolves in the muscle substance. The drag or frictional resistance of the myoplasm is reflected in the turbulent eddies and distortions of the cross striations produced at the prow and in the wake of the travelling neurosomes (for comparison with Figs. 63 to 75). Gold technic, teased whole muscle fibers. $\times 200$.

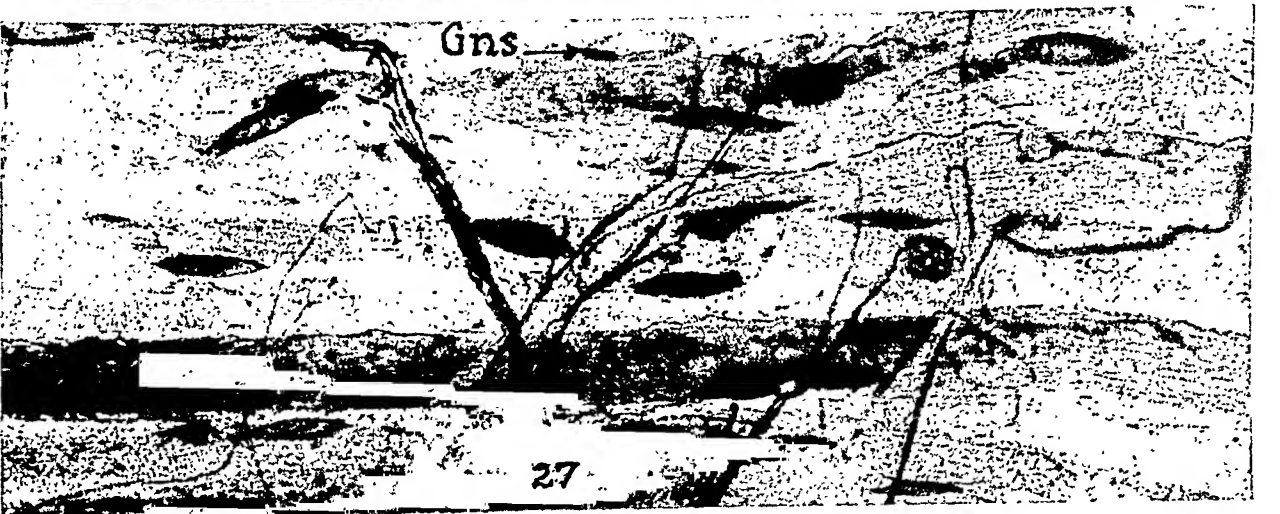
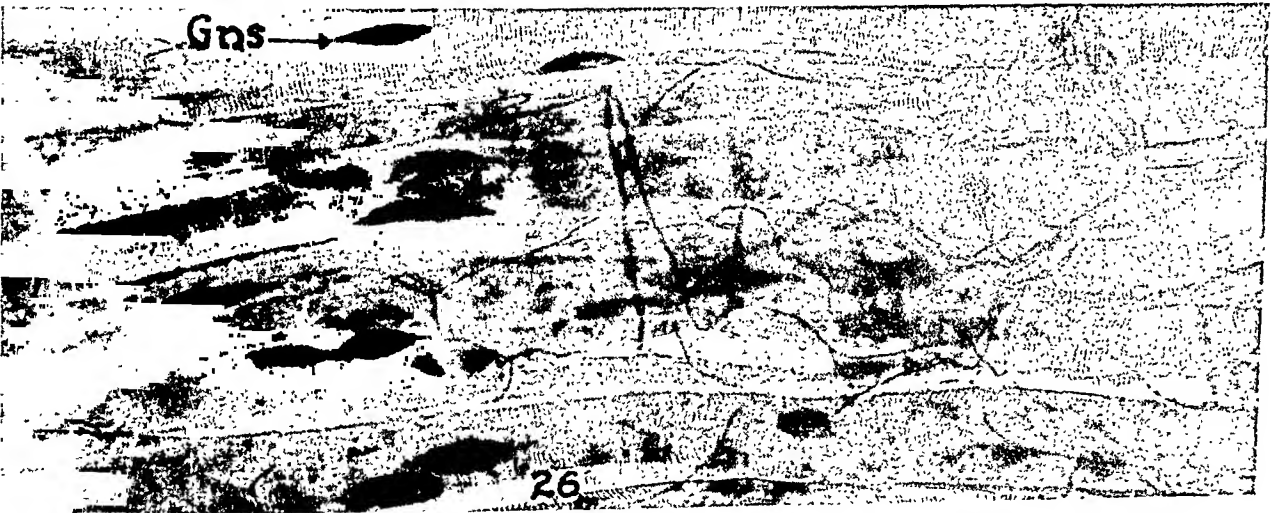
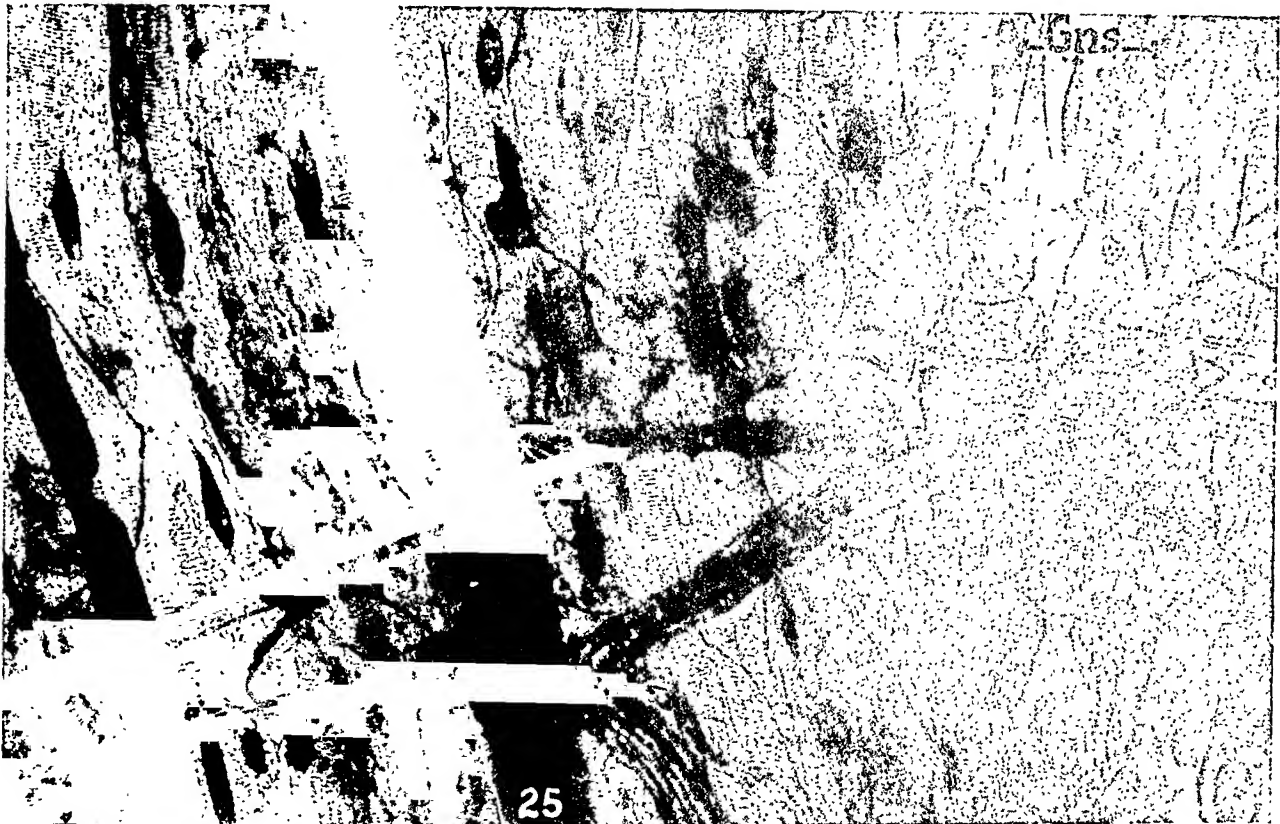


PLATE 34

FIGS. 28 to 39. Normal (Figs. 30 to 32, and 35 to 38) and abnormal (Figs. 28, 29, 33, 34, and 39) variations in the structure of the motor nerve endings and epilemmal axons in the gastrocnemius muscle of the rat. The pleomorphism of the nerve ending appears to be produced by the neuroprotoplasmic streaming of amoeboid motion of the terminal hypolemmal axons. The periodic discharge of normal granules of neurosomes produces the granules of the sole plate of Kühne (Kg). Some of these granules are produced also by the nuclei related to the normal nerve endings. These granules are usually increased in amount around the retracted endings and depleted around the expanded (Figs. 34 to 37) and exhausted (Fig. 38) endings. The engorgement and depletion of the epilemmal axons appear to result from the centrifugal conduction and peripheral discharge of neurogenic substances by microperistaltic waves. During the first week after tenotomy, the hypolemmal and epilemmal axons appear to be engorged with an increased amount of gold-impregnated material (Figs. 28, 29, 33, and 34). From the 7th to the 14th days, the motor end-plate usually takes a uniform impregnation with gold because of the increased amount of neurogenic material which it contains. In direct continuity with this pathologic nerve ending (Fig. 39, NS), there are projections of pathologic neurosomes which likewise are deeply impregnated with gold. Gold technic, teased whole muscle fiber. $\times 850$.



PLATE 35

FIGS. 40 to 46. Hyperchrysophilia of motor nerve endings in the gastrocnemius muscle of the rat 10 days after tenotomy. The densely impregnated nerve endings are spherical, oval, or fusiform. In some nerve endings there is a beginning discharge of a light cloud of granules (Figs. 43, 44, and 46) to the right of the nerve ending. The genesis of a giant neurosome is observed in the last nerve ending (Fig. 46, Gns). With silver impregnation after formalin fixation there is evident an increased number of nuclei around the motor end-plates as well as an increase in the number of the neurofibrils in the terminal axons. The significance of these changes manifested by silver is not evident without a comparison with the morphologic changes revealed by the teasing of whole muscle fibers after gold impregnation. The latter method is better than the former to demonstrate that the motor end-plate is a microscopic gland of internal secretion, the structure of which varies with the phases of secretion. Gold technic, teased whole muscle fibers. $\times 850$.

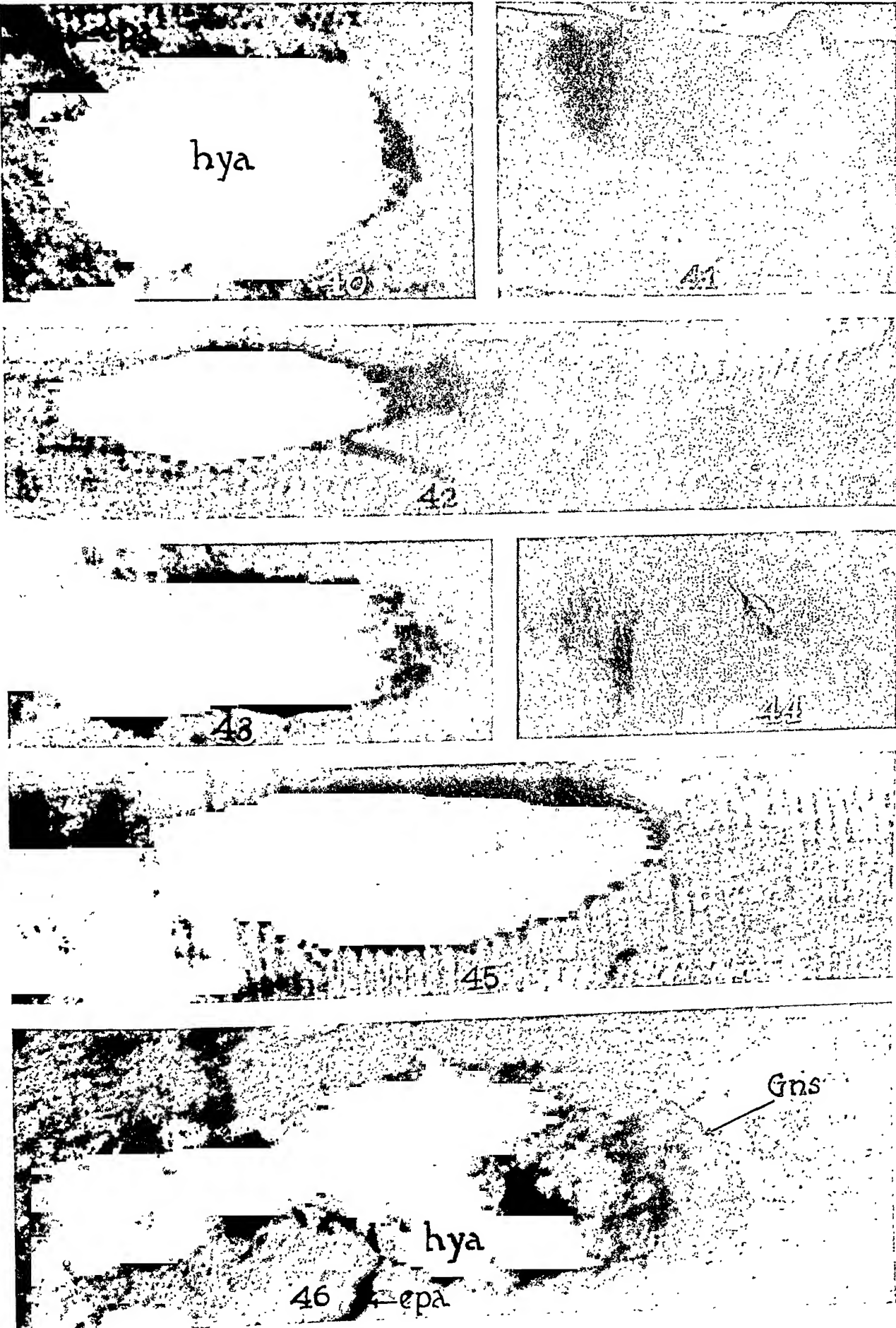


PLATE 36

FIGS. 47 to 56. The genesis of giant, globular and hemispherical pathologic neurosomes (Gns) from nerve endings in the gastrocnemius muscle of the rat 12 days after enclosing the right hind leg in a plaster of Paris cast. The mushroom eruption of the light cloud of granules from two nerve endings (Figs. 52 and 53) has a microscopic structure roughly comparable to the cloud of eruption produced by the explosion of the atomic bomb at Bikini in 1946. There is a complete transformation of some terminal nerve endings into a fusiform mass of granules (Figs. 55 and 56). The morphologic changes in muscle by immobilization of the limb in a cast are quite comparable to those produced by tenotomy. The neurosomic masses discharged during the early stages of disuse produced by a cast are usually more globular and oval than those produced subsequent to tenotomy. This mechanical deformation of the giant neurosomes may be dependent upon the differential rates of atrophy of muscle following the two methods of inducing disuse. Gold technic, teased whole muscle fibers. $\times 850$.

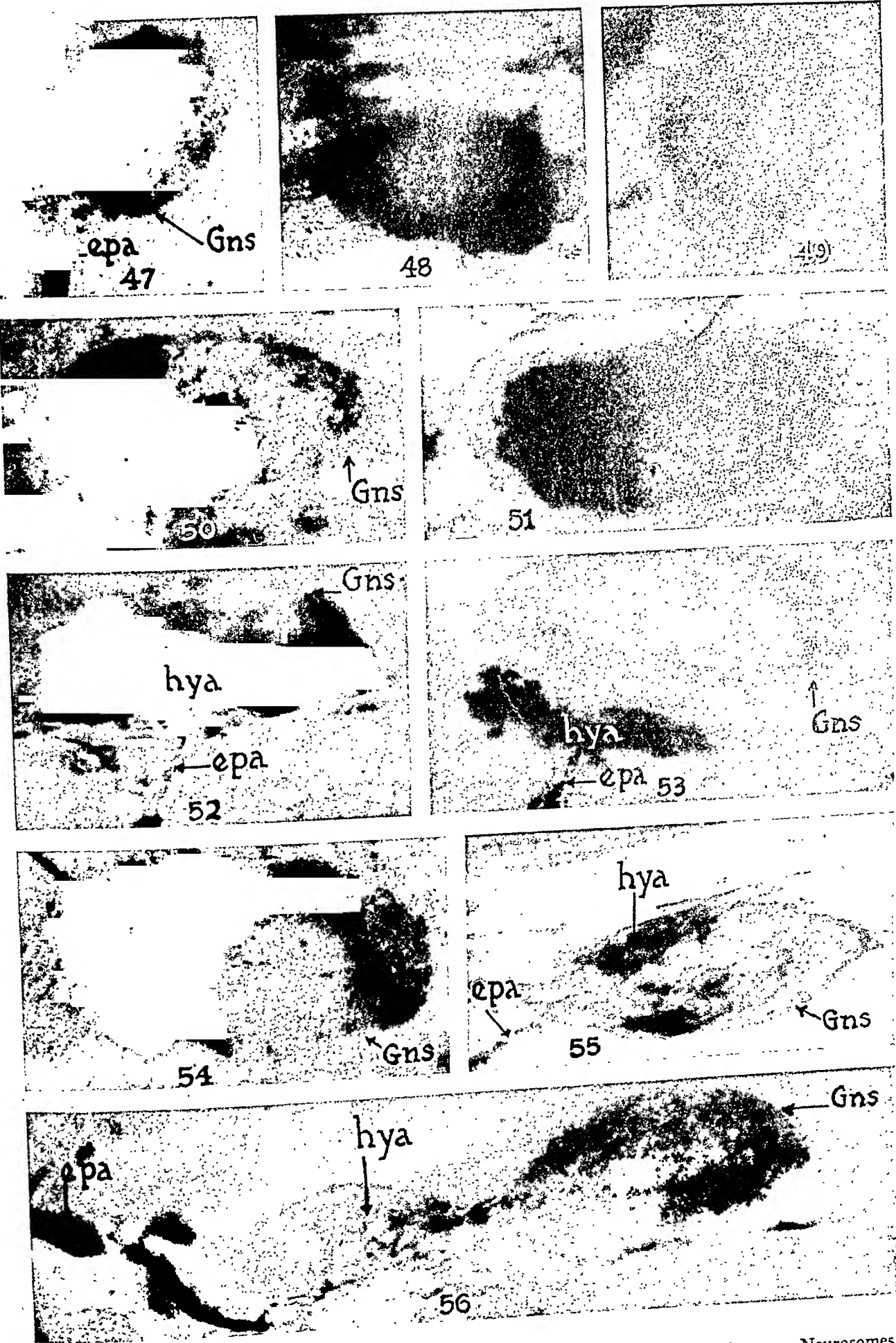


PLATE 37

FIGS. 57 to 62. The genesis of elongated, giant fusiform neurosomes (Gns) from motor nerve endings in the gastrocnemius muscle of the rat 15 days (Figs. 57 to 61) and 1 day (Fig. 62) after tenotomy. There are progressive stages in the accumulation, projection, and discharge of these neurosomes retarded in rate of secretion by the disuse of the muscle following tenotomy. Some of these neurosomes are intensely impregnated with gold, whereas others take the gold impregnation very lightly. The slowing in the rate of discharge of nerve impulses into muscle by disuse determines the increase in the quantity of the neurogenic secretion observed at and around the nerve terminal. The visualization by photography of the pathologic nerve impulse is, therefore, determined by the accumulation of neurogenic substances produced by the retardation in the rate of discharge, diffusion, and dissolution by hydrolysis of the neurogenic secretion from the motor nerve endings. Gold technic, teased whole muscle fibers. $\times 850$.

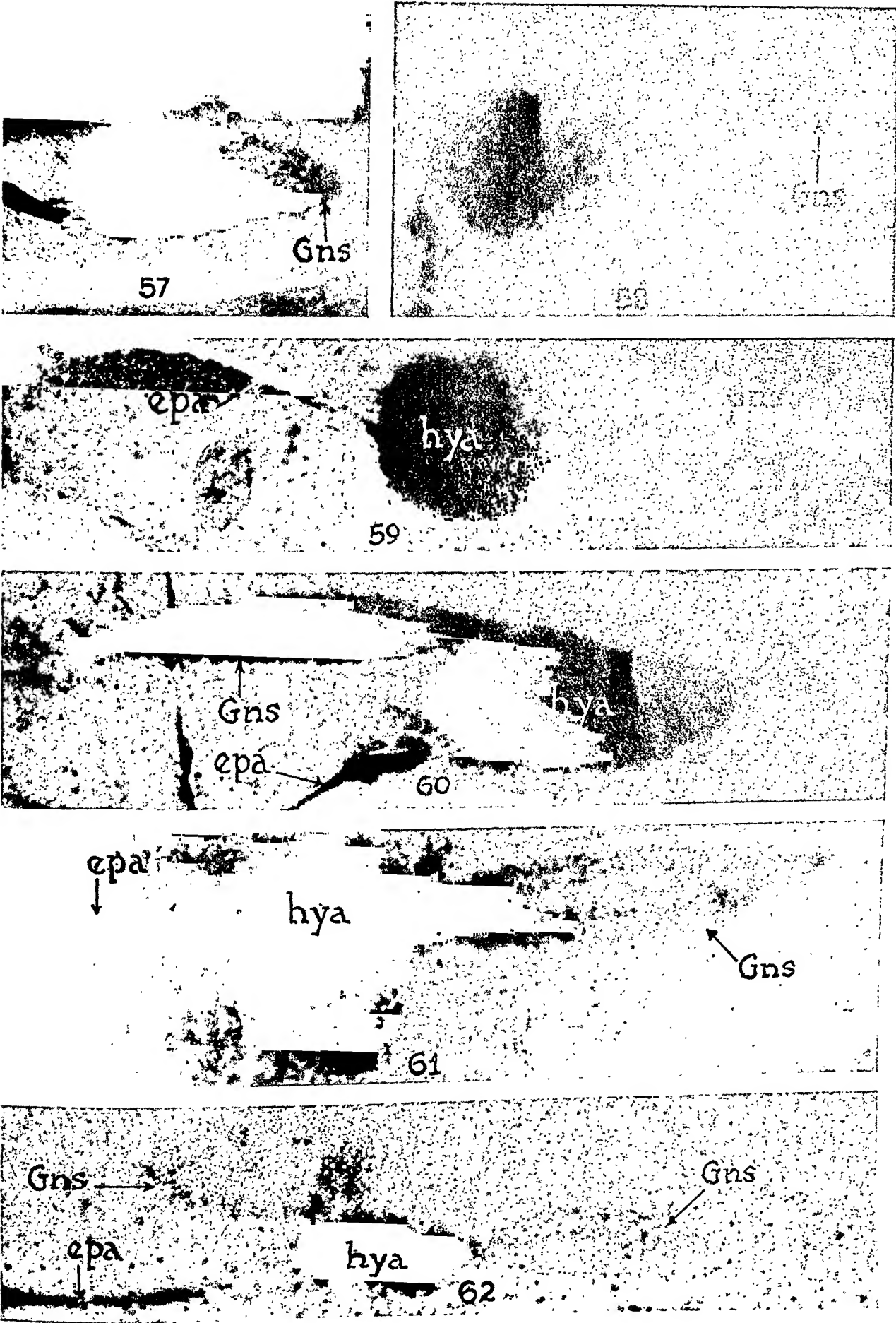
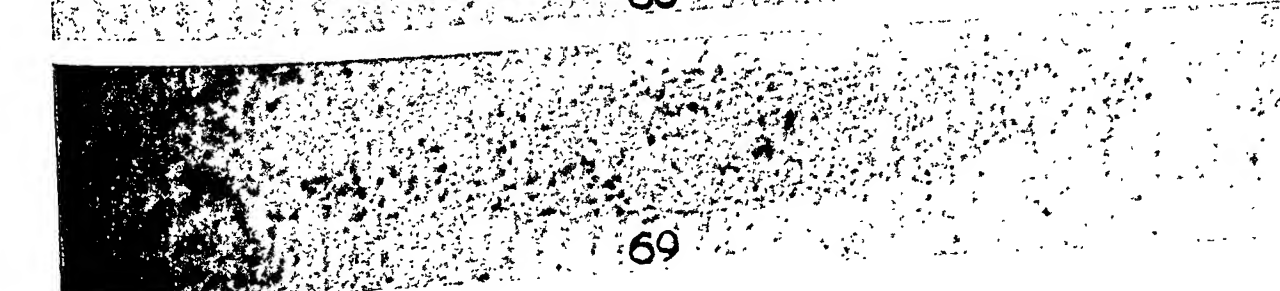
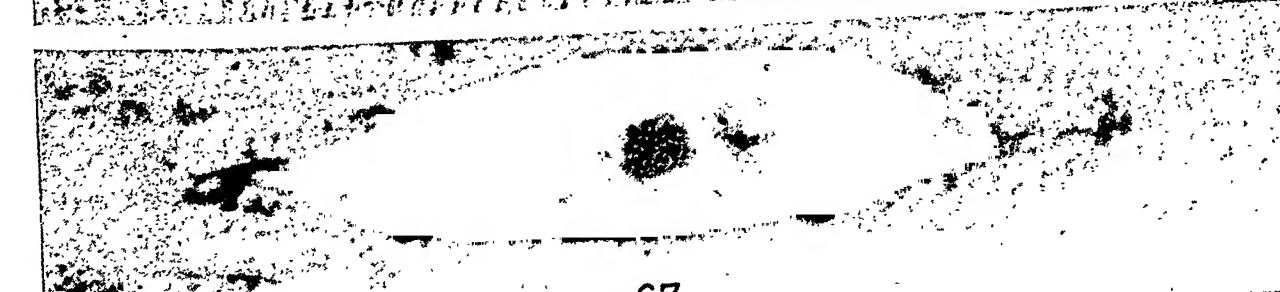
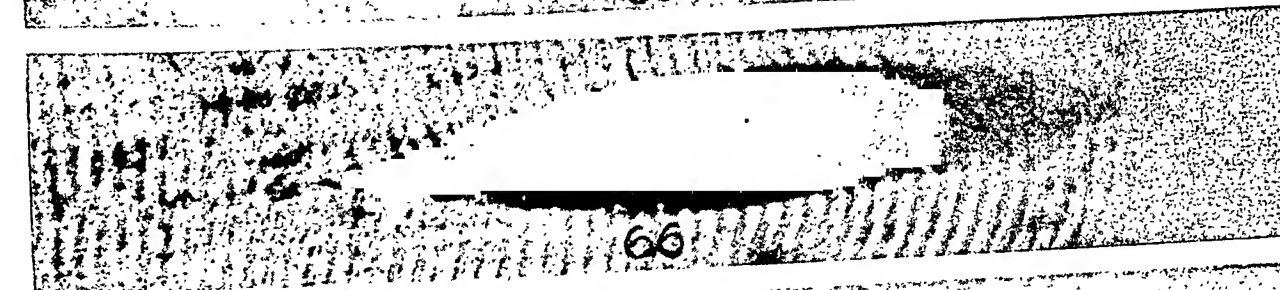
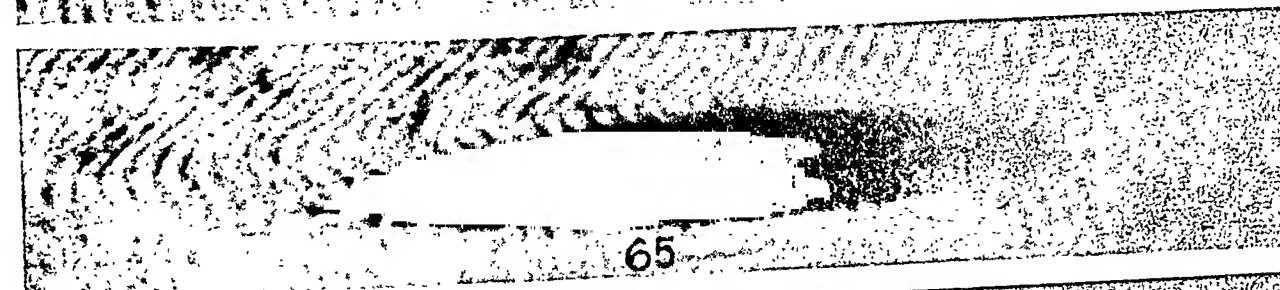
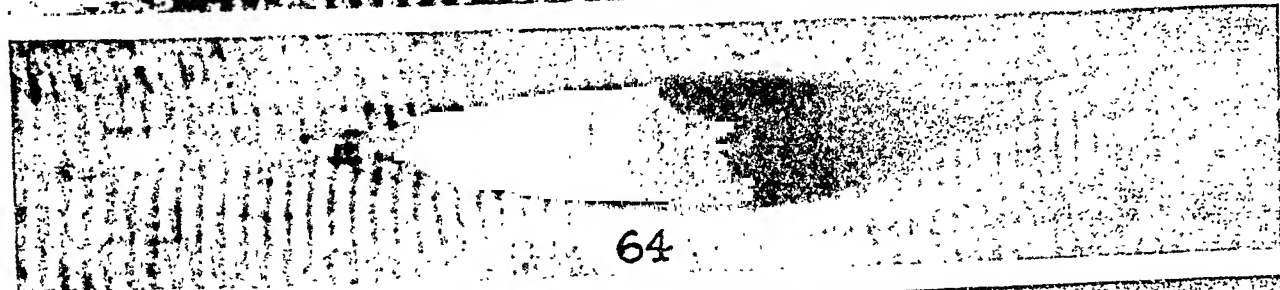
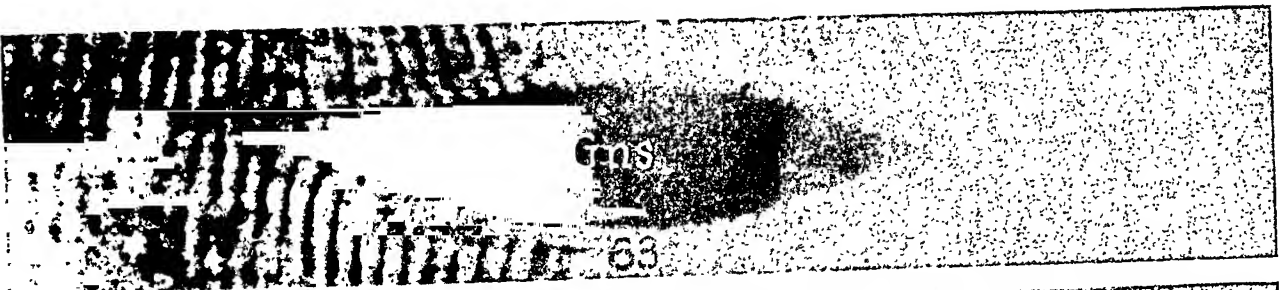


PLATE 38

FIGS. 63 to 69. Giant fusiform neurosomes (Gns) in the myoplasm of the gastrocnemius muscle fibers of the rat 15 days after tenotomy. Friction of the myogenic fluid slows down the transmission of the giant neurosomes. The resisting drag of the myoplasm on the moving neurosomes is associated with the formation of whirlpools or eddies. These turbulent eddies trail behind the neurosomes. The streamlining of the neurosomes results in tapering ends. The pointed rear end is more important in streamlining than the front end and in some locations (Fig. 65) neurosomes assume the "tear-drop design" of streamlining with one end blunt and convex like that of a torpedo. This design is modified, however, by the fact that the neurosome is not an inert particle moving in the muscle, but is subjected to colloidal interaction and undergoes progressive incorporation in the muscle. The resulting eddies or vortices are detected by the distortion of the related cross striations at the prow and in the wake of the giant neurosomes. These neurosomes undergo progressive dissolution (Figs. 68 and 69) by hydrolysis, and their granules become incorporated into, and aligned with, the cross striations of the muscle. Gold technic, teased whole muscle fibers. $\times 850$.

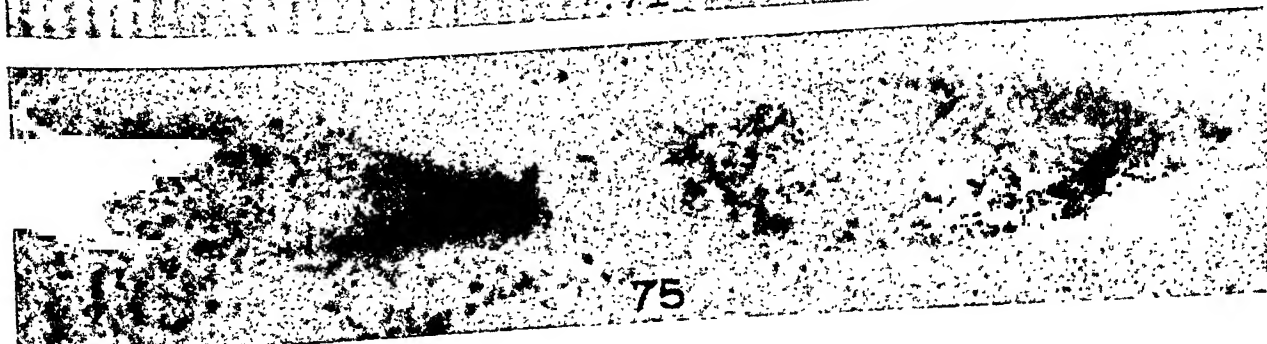
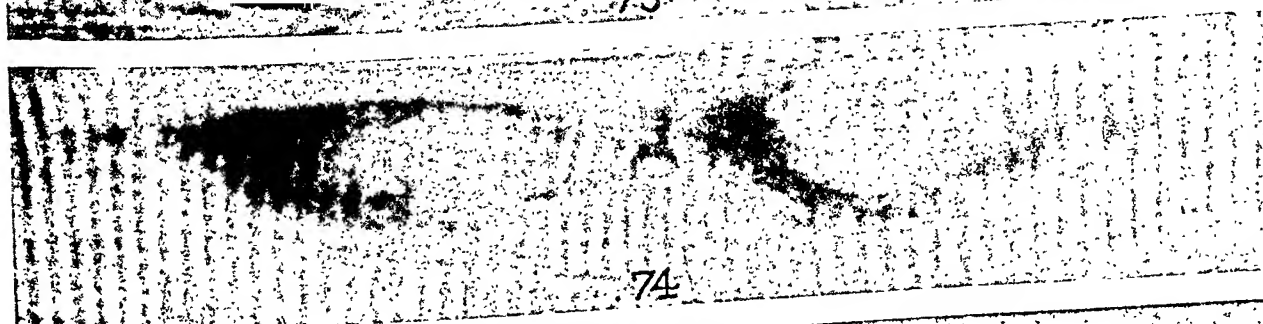
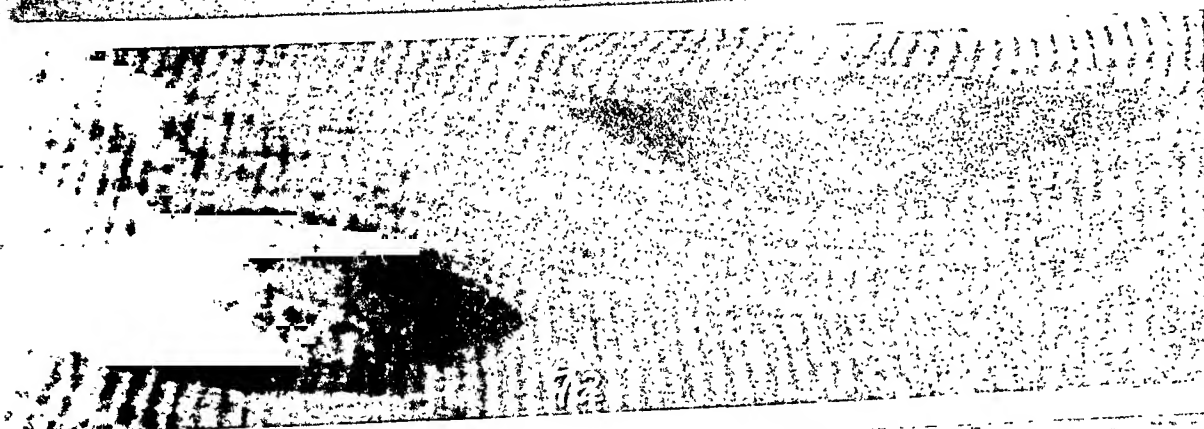
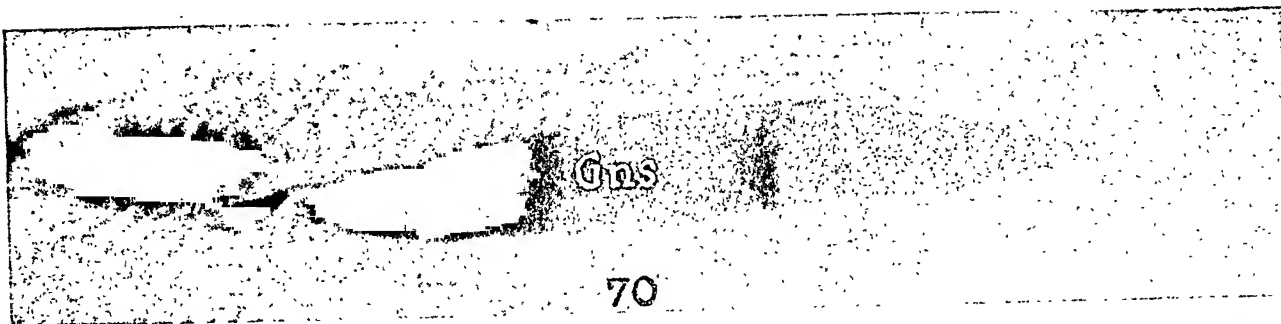


Effects of Disuse on Neurosomes

Carey, Haushalter, Massopust, *et al.*

PLATE 39

FIGS. 70 to 75. Single (Fig. 70) and duplex giant fusiform neurosomes (Gns, Figs. 71 to 75) in the gastrocnemius muscle of the rat 18 days after tenotomy. Some neurosomes are hyperchrysophilous (Figs. 70 and 71). Others, in different degrees of dissolution, are hypochrysophilous (Figs. 72 to 75). These neurosomes gradually disappear by hydrolytic granulation, and their granules become aligned with the striations of the muscle. The structural expression of these pathologic neurosomes in muscle is the resultant of a slowing down in the rate of diffusion and dissolution of neurosomes accompanying the retardation in rate and consequent increase in mass of the neurosomes that parallel the pathologically slow rate of nerve impulses in muscle undergoing atrophy of disuse. The real function and significance of the structure of the normal neuromuscular apparatus are revealed by retarding the rate of the normal discharge, diffusion, and dissolution of the neurosomes that accompany the normal electric signs of the nerve impulses. Gold technic, teased whole muscle fibers. $\times 850$.



VASCULAR PROLIFERATIONS, WITH FEATURES OF ARTERIOVENOUS ANASTOMOSES, IN THE SYMPATHETIC CHAIN OF HYPERTENSIVE PATIENTS *

RAFFAELE LATTES, M.D.

(From the Department of Pathology of the New York Post-Graduate Medical School and Hospital and Columbia University, New York, N.Y.)

In the course of the routine histologic study of large numbers of thoracolumbar sympathetic ganglia removed surgically in patients with essential hypertension, peculiar vascular structures of organoid appearance were observed repeatedly. It is the object of this paper to describe these findings, and to discuss their possible significance.

MATERIAL

The observations reported here have been made on specimens from thoracolumbar sympathectomies performed on hypertensive patients. The portions removed usually included the area from the second or third thoracic ganglion to the second or third lumbar ganglion. However, because the specimens were not always removed in one piece, a proper identification of the provenance of an individual ganglion was often impossible. As control material, the sympathetic chains of 5 patients who had died from diseases other than essential hypertension were obtained at autopsy. The respective ages of these patients were 2 days, and 11½, 26, 35, and 52 years. For the majority of the cases, the ganglia were fixed in Zenker's solution, and the paraffin sections were stained with hematoxylin and eosin, Masson's trichrome stain, resorcin stain for elastic fibers, and Wilder's stain for reticulum. A few of the specimens, after fixation in chloral hydrate, were stained with the Cajal silver nitrate method. Some were fixed in Orth's fluid and stained with the method of Schmorl. In 8 cases, all of the ganglia removed by the surgeon were studied in serial sections. In general, however, the paraffin blocks were cut at three or four different levels, but no complete serial study was made.

MICROSCOPIC FINDINGS

In a fraction of the cases examined, the sympathetic ganglia or the immediately adjacent nerve trunks showed the presence of well defined, usually round bodies, up to 250 μ in approximate diameter, situated either in the substance or near the capsule of the ganglion,

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Read at a meeting of the New York Pathological Society, October 30, 1947.

and consisting of a complex system of narrow, tortuous blood channels arranged in glomerular* fashion, associated with an arteriole and connected with efferent venous sinusoids (Figs. 1 to 7). The small vessels of these vascular bodies were lined by large endothelial cells that could be remarkably swollen so that the respective lumina often were inconspicuous. The more or less abundant intervening stroma contained varying numbers of elongated or round elements with oval, vesicular nuclei larger than those of the adjacent endothelial cells. The cytoplasm of these extra-endothelial cells as a rule was clear; it might contain small eosinophilic granules but no definite myofibrils. These extravascular elements were at times (but not always) wrapped around part of the circumference of the small vessels of the bodies. In other instances they appeared to be scattered in the stroma between the vascular loops.

The arteriole associated with these structures showed, in general, a marked hypertrophy of its muscular coat, and often hyalinization and degeneration, even to a marked degree. In most of the cases, however, the small vessels of these structures seemed to sprout directly from the wall of the associated arteriole and to communicate with its lumen (Figs. 6 and 7). Schmorl stains for chromaffin substance have been negative in these bodies, and no relationship appears to exist between them and the not infrequent finding of masses of chromaffin tissue in the vicinity of the ganglia. The study of a possible innervation of these small organoid structures has been difficult because to date I have found no way of predicting, through clinical data or gross inspection, which of the ganglia will show positive findings. However, after sectioning serially several ganglia prepared with the Cajal method, some vascular glomeruli were identified. The photomicrograph (Fig. 5) shows that the silver impregnation has been successful but that the body itself contains no axons. Regressive changes have been observed in some of the glomerular structures, and they consist in general of foci of fibrinoid degeneration and necrosis similar to those observed in the walls of the arterioles in malignant hypertension. These are interpreted as diseased arteriolar segments.

As has already been mentioned, the microscopic findings in some of the cases suggest that the tortuous capillaries and the intervening extra-endothelial cells (pericytes?) composing these glomerular vascular structures take their origin from the adventitia of diseased arterioles. These appear to be surrounded by a highly cellular zone, in

* The term "glomerulus" or "glomerular structure" used here and in the following paragraphs is not intended to imply any resemblance to the renal glomerulus, nor to the neuromyo-arterial glomus. It is used in its original literal meaning of "small body."

which there are narrow capillaries and large irregular cells with vesicular nuclei, similar to those seen, and already described, in the fully developed vascular bodies (Figs. 8 and 9).

Furthermore, in some recent favorable cases in which the vascular glomerular structures could be studied three-dimensionally by means of serial sections, it appeared that some of the associated arterioles were segmentally involved by severe necrotizing arteriolar sclerotic disease, and that the proliferation of small blood vessels and accompanying pericytic cells was originating from the segments of the arteriole immediately adjacent to the involved portion.

Before ending this description, mention should be made of the venous circulation in the sympathetic ganglia. This appears to consist exclusively of sinusoidal vessels, with prominent endothelial lining, and no muscular coats. These sinusoids are often seen to be situated alongside of, and in direct contact with, the muscular coats of the adjacent small arteries and arterioles. It is in these venous sinusoids that the blood from the capillary system of the ganglion is collected (Fig. 10). The existence of similar large venous sinusoids in the sympathetic ganglia of rabbits was recognized and described by Ranvier in 1875. The probable importance of this type of sinusoidal circulation in the mechanism of formation of the vascular glomerular structures will be discussed below.

INCIDENCE

Observations were made from specimens from 95 thoracolumbar sympathectomies. Vascular glomerular structures were found in 9 patients, in 3 of which the bodies were found in the ganglia of each side. Ganglia containing multiple bodies were found four times: two contained two bodies, one four, and another five. These findings make no pretense of statistical accuracy, as serial sectioning was used only in a few of the specimens.

RELATIONSHIP BETWEEN PRESENCE OF VASCULAR BODIES AND CLINICAL FEATURES

The 9 patients in whom the vascular glomerular structures were found were all in advanced stages of arteriolar sclerotic hypertension. The average age was 35.11 years. There were 7 females and 2 males. The average duration of the disease was 7.5 years. All of the patients complained of subjective symptoms consisting of headaches, nervousness, dizziness, and often blurred vision. In 7 the eyegrounds showed sclerosis of the arterioles, arteriovenous nicking, and old and recent hemorrhages. In 2, no record of ophthalmologic examination was found in the clinical chart. At least traces of albumin were found in the urine

of all. The urea clearance, and the pitressin concentration tests showed definitely impaired renal function in 4, and apparently in only one case was the function of the kidney within normal limits.

DISCUSSION

The objects under consideration are well defined, complex vascular bodies of organoid structure which appear to be interposed between vessels of arterial and of venous type. The associated arteriole is in general affected by advanced arteriolar sclerosis. Between the small tortuous capillaries of the "glomeruli" there are varying numbers of large, clear, extra-endothelial elements resembling the pericytes studied originally by Zimmermann and more recently by Murray and Stout (Figs. 2, 8, and 9). Some of the histologic findings seem to indicate that the vascular elements in question originate from a proliferation of small blood vessels and of pericytic cells which, starting in the adventitia of diseased arterioles, connects them with large venous sinusoids.

To the best of my knowledge, no similar structures have hitherto been described, either for normal or pathologic human sympathetic ganglia. Furthermore, the personal study of serial sections of the stellate ganglion and of the thoracolumbar sympathetic ganglia of 5 patients dying from causes other than essential hypertension failed to reveal any comparable finding. Only one paper could be found in the available literature which appeared to offer a possible lead in the interpretation of these findings. This is an article by Nonidez, in which he described in great detail his observations of simple and multiple arteriovenous anastomoses in the stellate and thoracic ganglia of young dogs, believed by him to belong in the same class with the glomus coccygeum and the peripheral neuromyo-arterial glomus of Masson. These are organoid structures consisting of multiple arteriovenous channels surrounded by conspicuous clear epithelioid cells which are richly innervated by efferent and afferent fibers. Nonidez suggested that they may represent organs for the regulation of the blood pressure. No similar structure appears to exist in the normal human sympathetic ganglia.

The structures described in this paper and the bodies of Nonidez in the dog appear to have only a superficial resemblance. Both have an organoid architecture and consist of a complex network of blood vessels interposed between an arteriole and efferent venous sinusoids. However, in the structures described by Nonidez, the vascular channels of the multiple anastomoses show orderly arranged, perivascular, clear epithelioid cells interpreted by him as modified smooth muscle cells. In the structures illustrated in this paper, extra-endothelial cells of peri-

cytic type are present, but are not as conspicuous nor arranged in as orderly a fashion. Furthermore, the rich innervation described by Nonidez in the structures studied by him could not be demonstrated in my cases. This seems to me to be a strong point against considering the present findings as structures of the neuromyo-arterial glomus type.

In my opinion, the occurrence of these vascular bodies is closely related to the presence of severe arteriolar sclerotic disease. In fact, this is strongly supported by some of the findings illustrated in the preceding paragraphs, and by the absence of similar structures in the ganglia of nonhypertensive persons. The vascular channels of these glomerular formations appear to represent a short cut between the diseased arterioles and groups of large venous sinusoids. Because of these features, they can be rightly considered as arteriovenous anastomoses, occurring in areas where the normal blood flow has been interfered with by pathologic changes in the local arteriole. That new arteriovenous anastomoses can appear in regions subjected to various types of irritation with disturbance of the blood flow has been conclusively demonstrated by Clark. Under the conditions described in my material, the appearance of arteriovenous anastomoses may represent an attempt to shunt the blood into the adjacent large sinusoids which constitute the venous system of the ganglia, and from here to redistribute it to the ganglion itself, through the numerous capillaries which open into these sinusoidal vessels (Fig. 10). Therefore, the observations of Nonidez on the dog acquire interest in the discussion of my findings. In fact, the presence of complex arteriovenous anastomoses in the sympathetic ganglia of other vertebrates may lead one to speculate on the existence of analogous but rudimentary structures in the human sympathetic ganglia from which the vascular bodies described above are derived. If morphologic peculiarities in the blood vessels of human sympathetic ganglia could be demonstrated, the presence of the vascular bodies in the ganglia and their absence in other organs or tissues of hypertensive persons could be understood. Search for such structures should be made either with injection technics, or with a three-dimensional model of the blood vessels of this region.

SUMMARY

Peculiar vascular proliferations, with features of arteriovenous anastomoses, have been observed in the thoracolumbar sympathetic ganglia of patients with severe essential hypertension. Their occurrence in sympathetic ganglia, and not in other organs or tissues of the hypertensive patient, is possibly related to some as yet unknown structural specialization of the blood vessels of this region.

I wish to express my gratitude to Dr. Maurice N. Richter and to Dr. Arthur Purdy Stout for their helpful suggestions and constructive criticism in the compilation of this paper. I wish also to express my thanks to Miss Evelyn Paget for her skillful histologic preparations.

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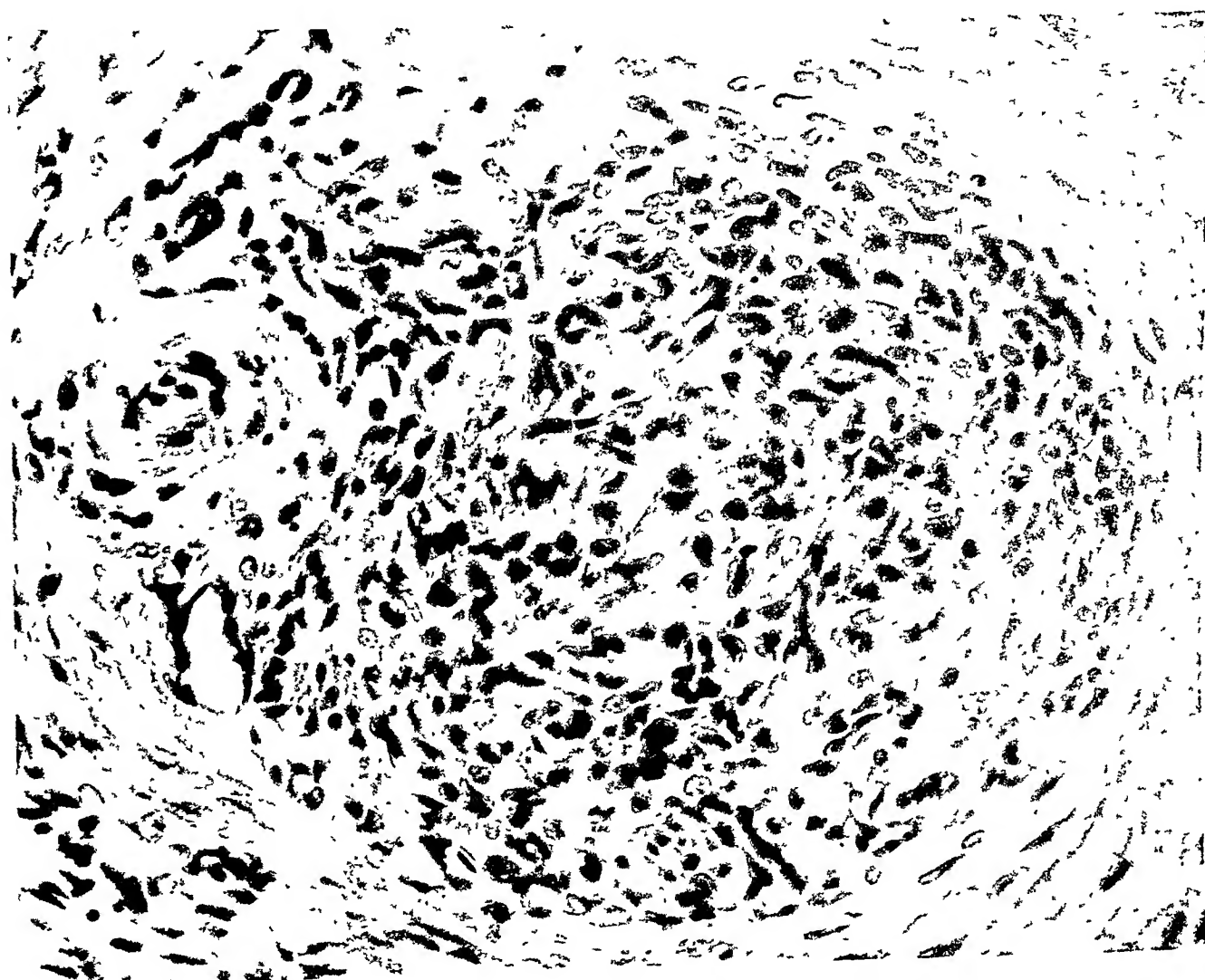
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DESCRIPTION OF PLATES

PLATE 40

FIG. 1. Photomicrograph showing two vascular glomerular structures near the capsule of a sympathetic ganglion from a case of essential hypertension. Hematoxylin and eosin stain. $\times 115$.

FIG. 2. Higher magnification of one of the "glomeruli" seen in Figure 1. On the left side may be seen an arteriole surrounded by clear, large, smooth muscle elements and three large venous sinusoids into which some of the narrow vessels of the vascular body open. Of note also are the numerous extra-endothelial cells between the vascular loops of the body. Hematoxylin and eosin stain. $\times 550$.



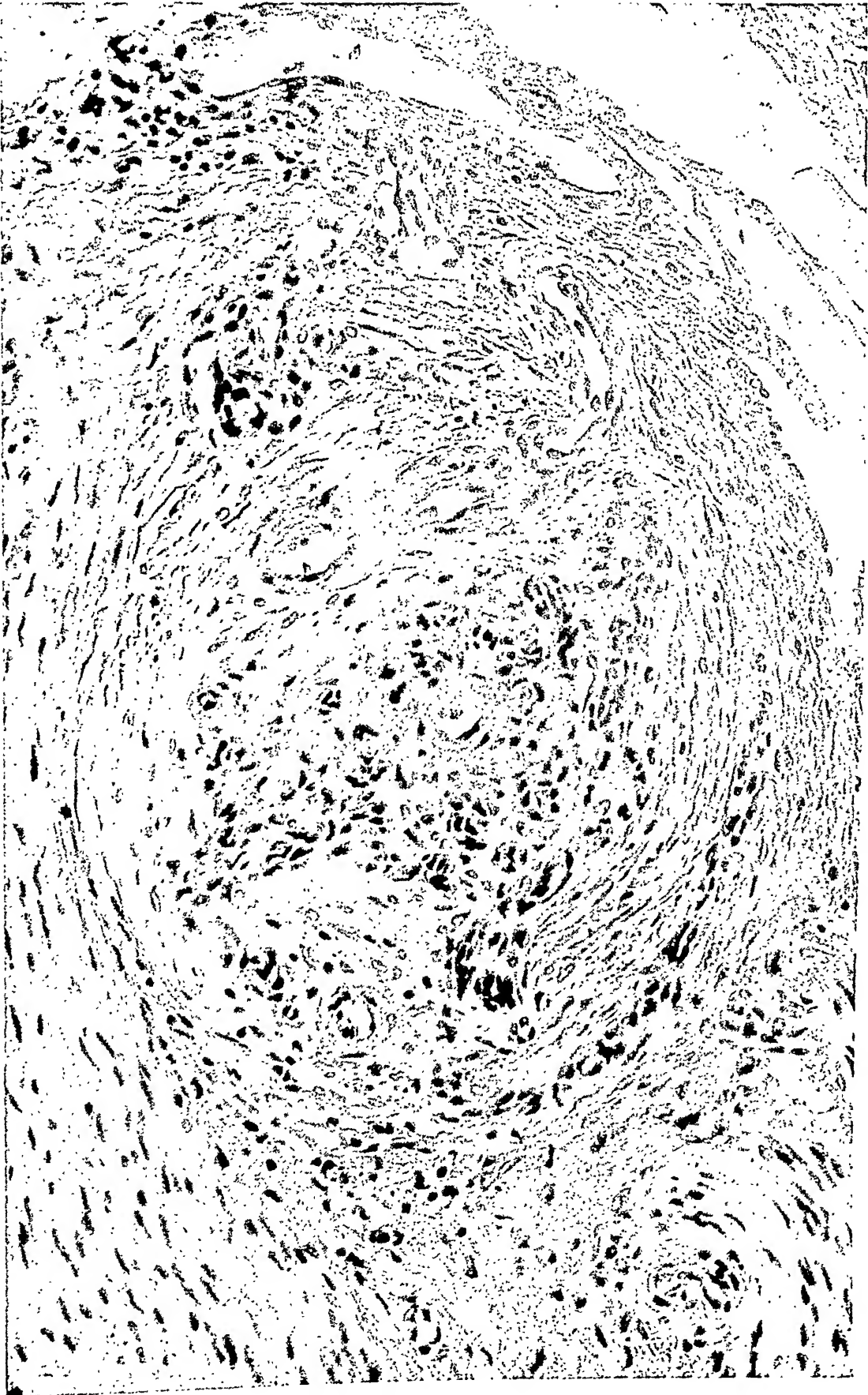
Lattes

Arteriovenous Anastomoses in the Sympathetic Chain

PLATE 41

FIG. 3. Glomerular structure at the periphery of a sympathetic ganglion, showing a well defined expansion zone of the stroma. Several cross sections of a sclerotic arteriole, and a group of vessels, some of which are surrounded by pericytic elements, may be seen streaming toward a large thin-walled vessel in the upper portion of the photomicrograph, probably the collecting vein of the "body." Hematoxylin and eosin stain. $\times 300$.

3



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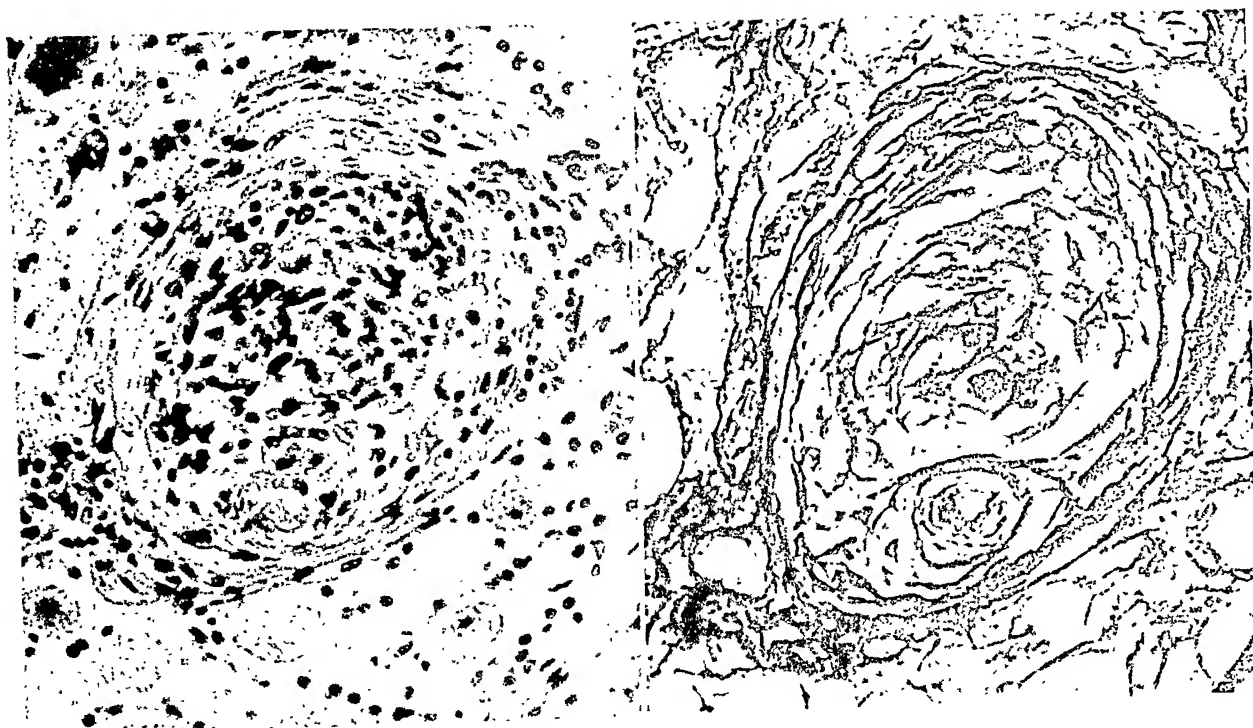
Arteriovenous Anastomoses in the Sympathetic Chain

PLATE 42

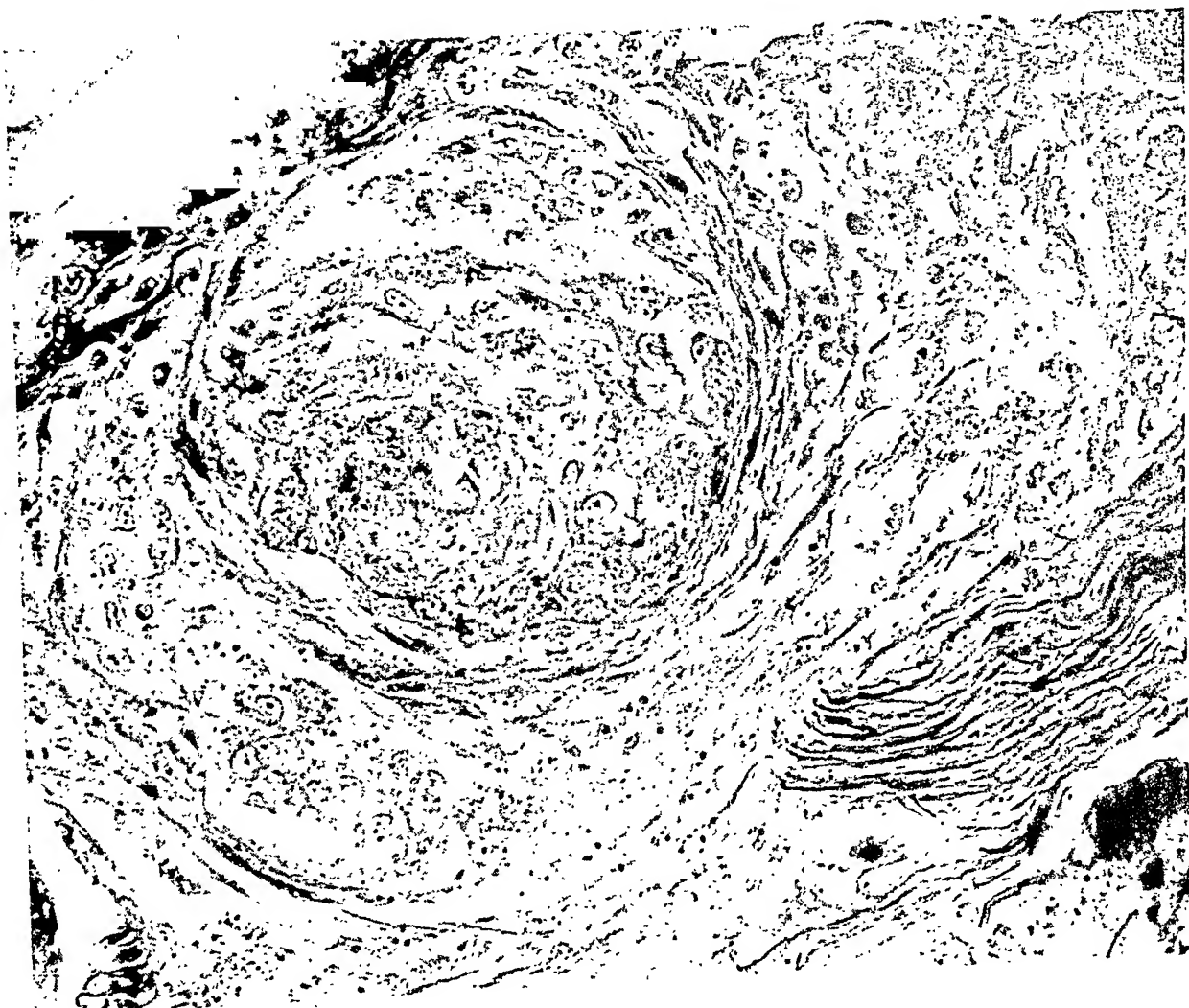
FIG. 4. Photomicrograph of two succeeding sections, stained respectively with hematoxylin and eosin and Wilder's silver stain for reticulum, of a vascular body found in the center of a ganglion. Of note are the complete encapsulation of the body and the partial hyalinization of the associated arteriole. The silver stain demonstrates the regular pattern of the reticulum. $\times 255$.

FIG. 5. Vascular glomerular structure in a sympathetic ganglion prepared by the method of Cajal. Axons are absent in the vascular body. $\times 500$.

4



5

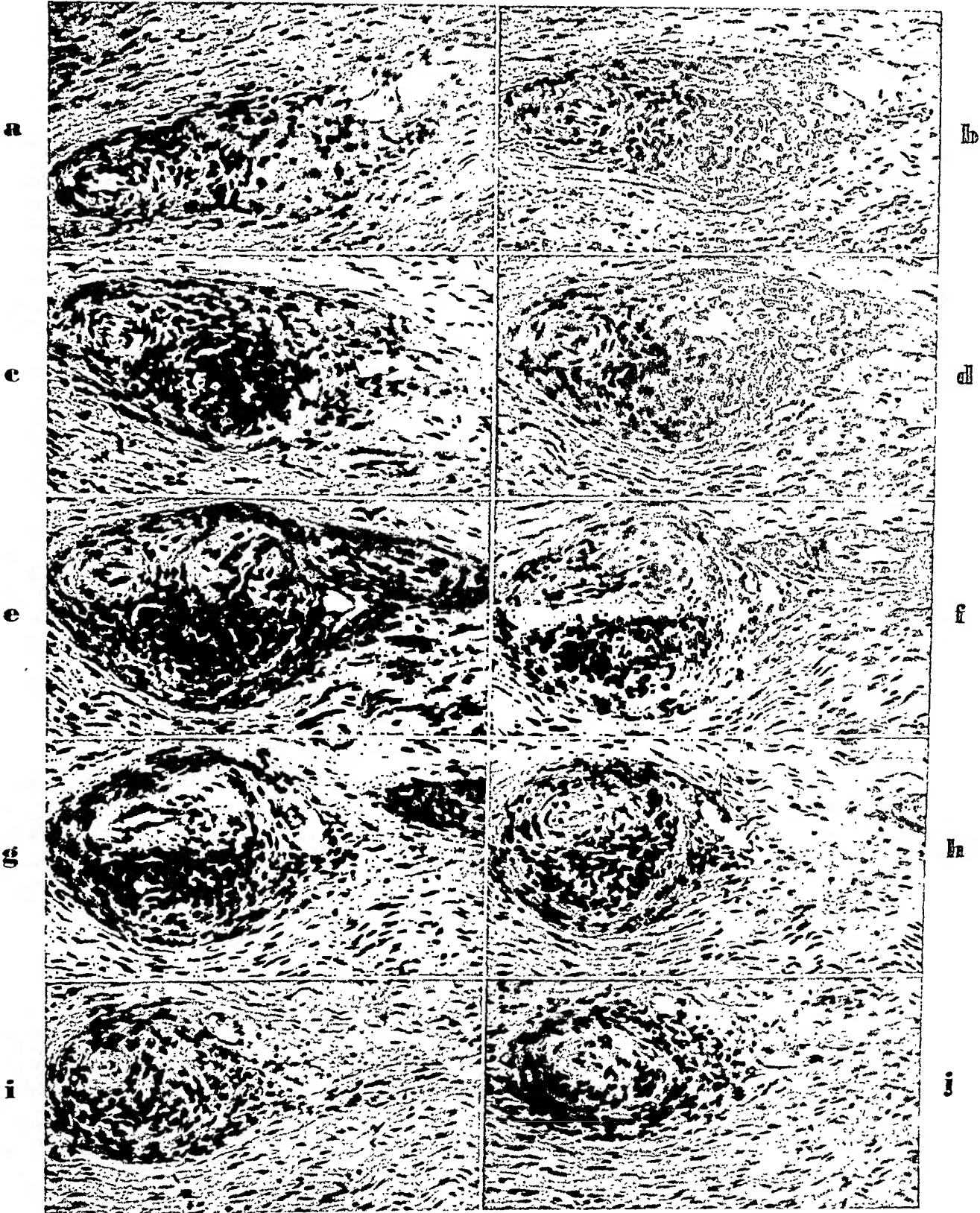


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Arteriovenous Anastomoses in the Sympathetic Chain

PLATE 45

FIG. 6. Composite picture showing every third section of a vascular body studied serially. There is a close relationship between the wall of the associated, tortuous, thick-walled arteriole and the vascular body. Section *c* clearly demonstrates a communication between the lumen of the arteriole and the vascular network of the body. (For additional details of this section see Fig. 7.) Of note also are the venous sinusoids at the periphery of the vascular body, into which the vessels of the body can be seen to open at different levels. Hematoxylin and eosin stain. $\times 140$.



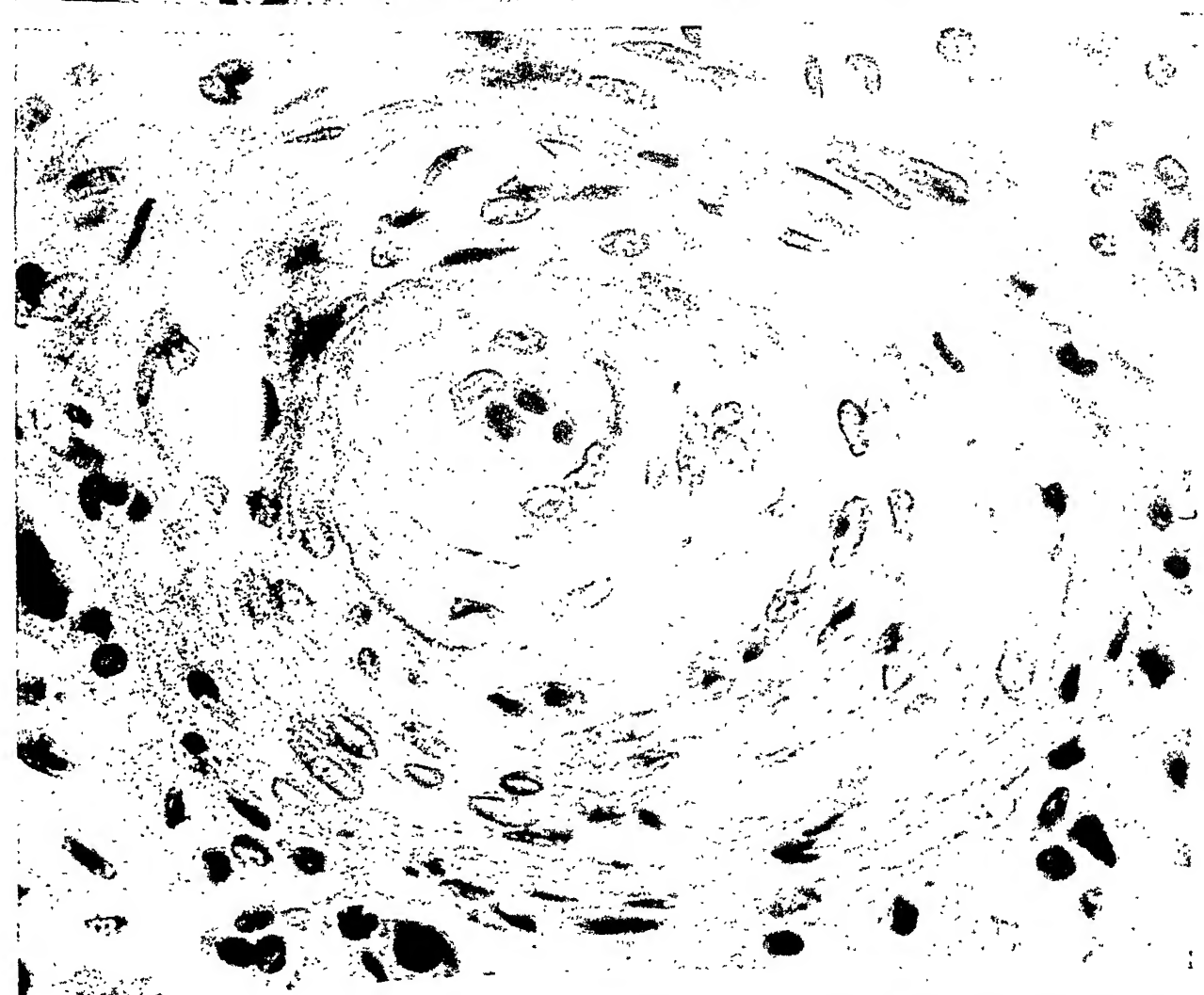
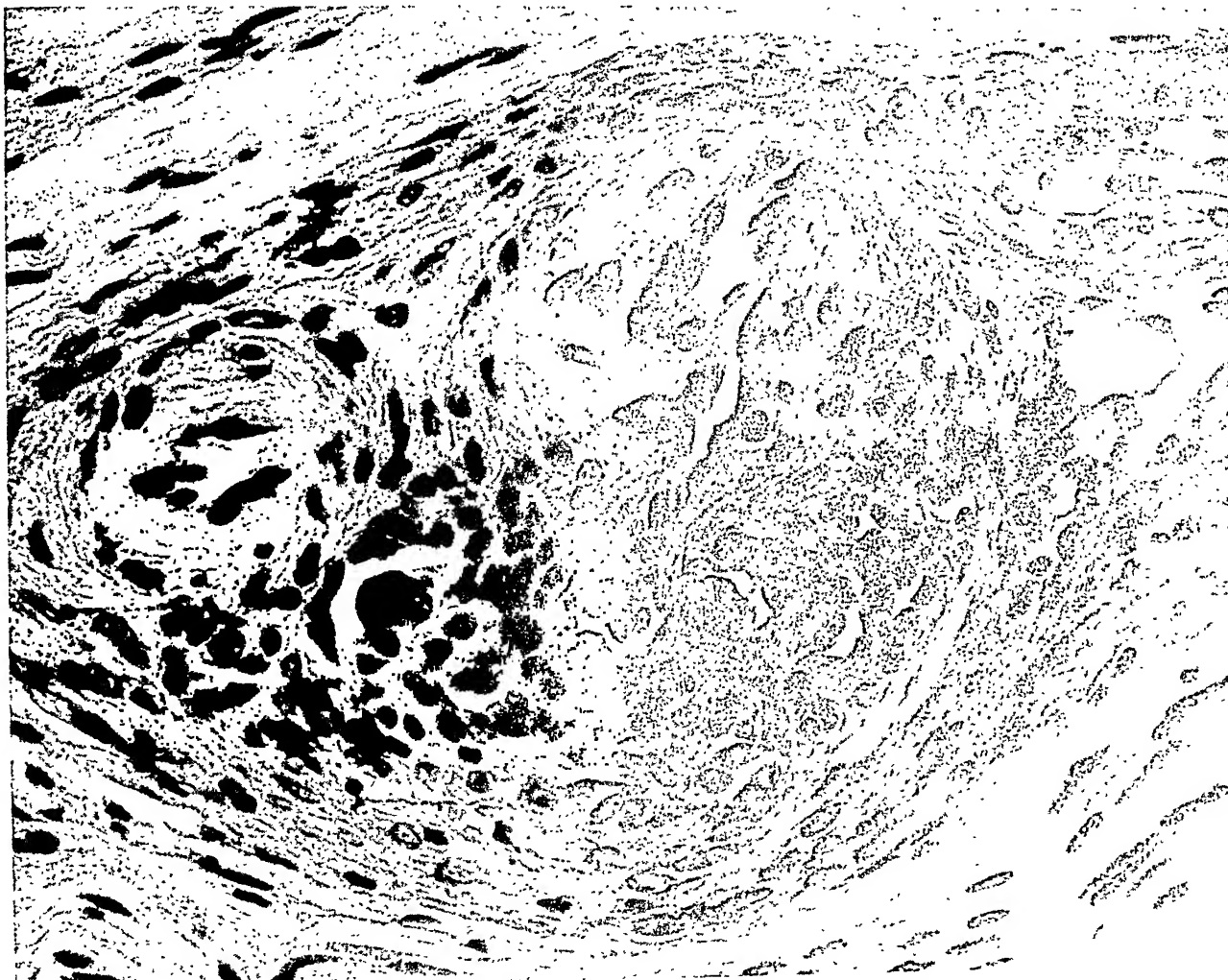
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Lattes

Arteriovenous Anastomoses in the Sympathetic Chain

PLATE 44

- FIG. 7. High-power photomicrograph of section *c* from the series shown in Figure 6. The glomerular body communicates with the lumen of the arteriole (center of field), and also with a peripheral venous sinusoid (right side of field). Hematoxylin and eosin stain. $\times 420$.
- FIG. 8. Photomicrograph of an arteriole in a sympathetic ganglion from a case of essential hypertension. The marked concentric thickening apparently is caused to a great extent by the presence of large numbers of adventitial cells. Also, in the peripheral zone there is formation of small spaces lined by flattened cells of endothelial appearance. This may be an early stage in the histogenesis of the glomerular vascular structures. Hematoxylin and eosin stain. $\times 650$.



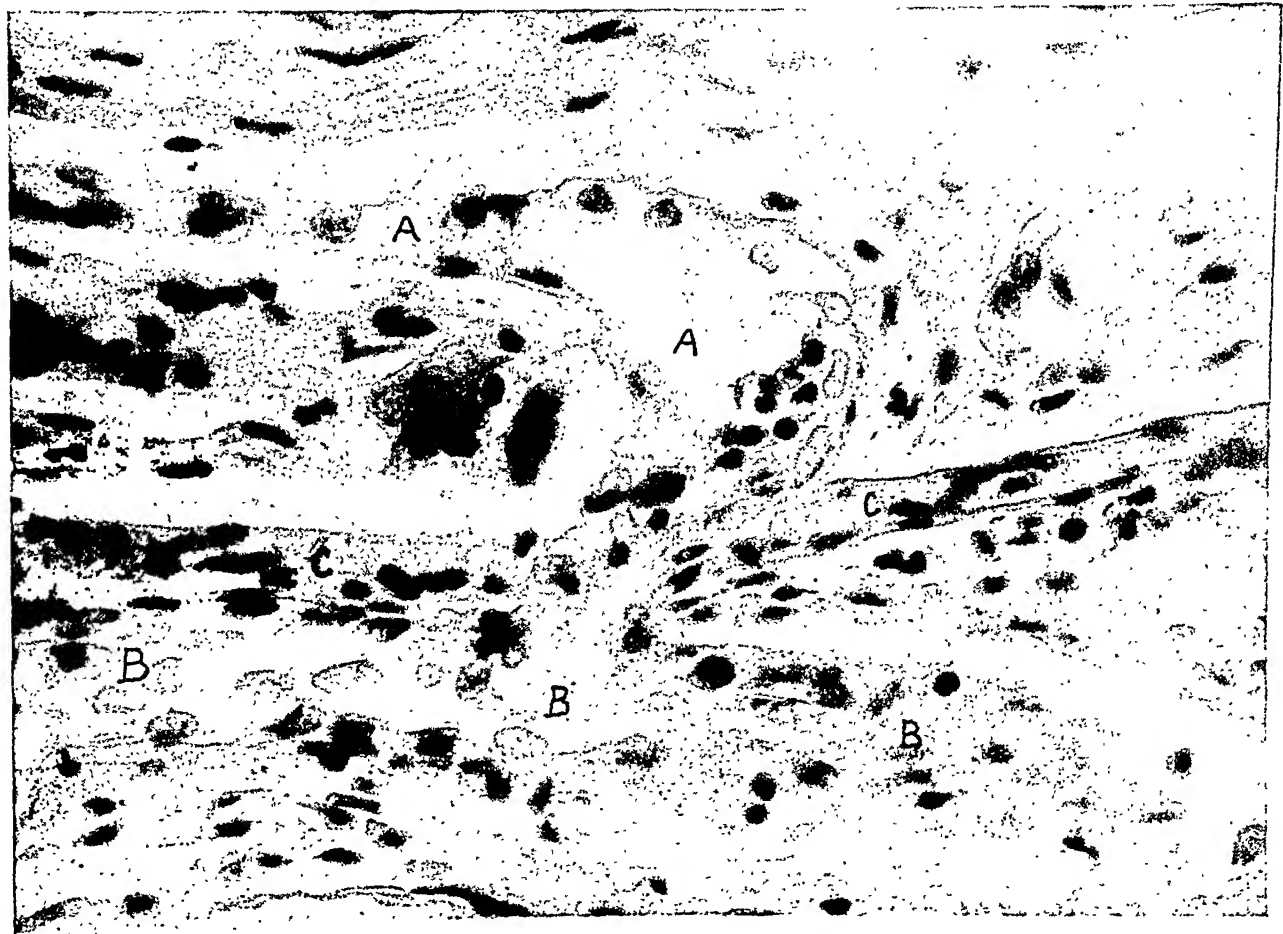
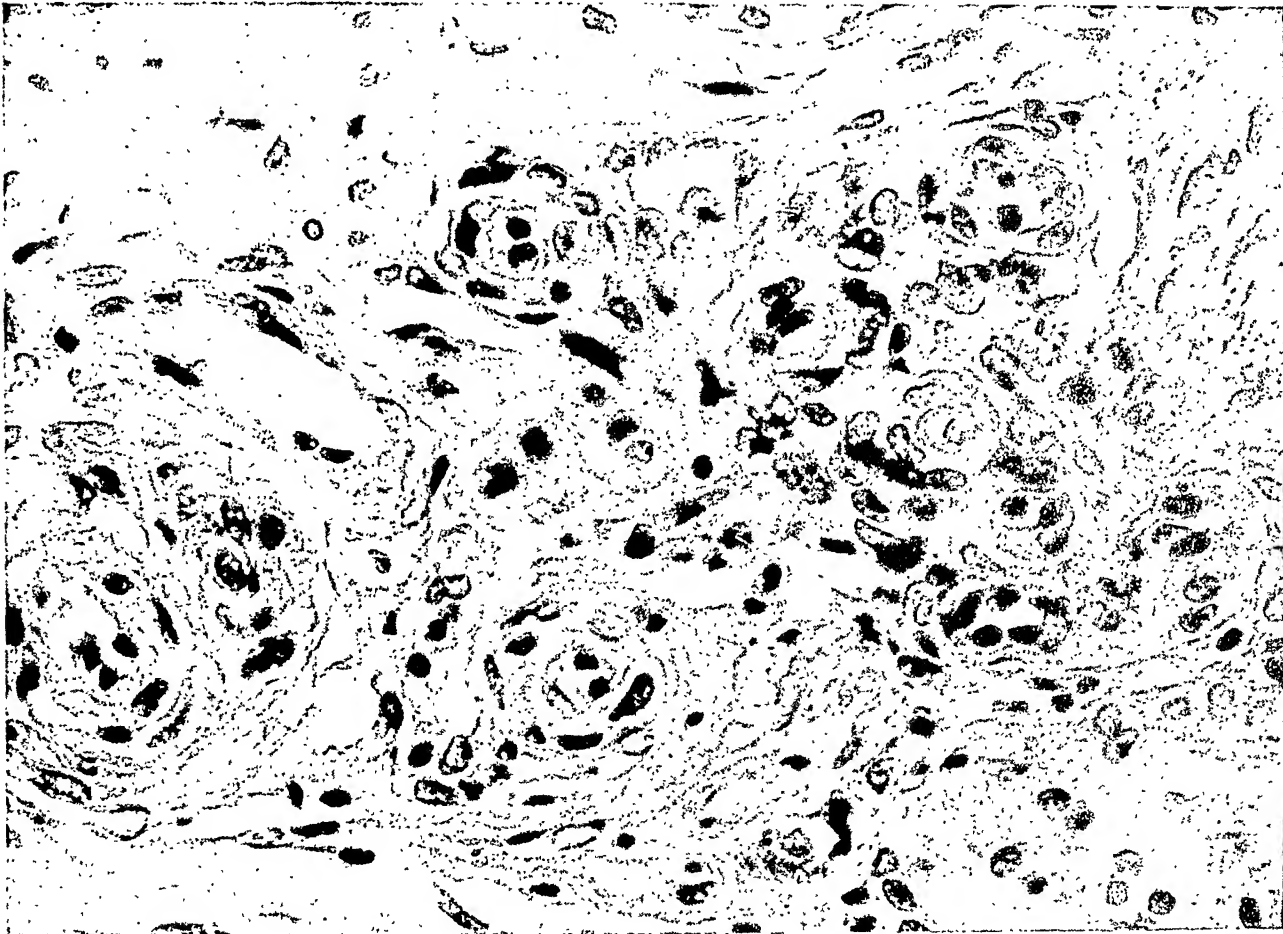
Arteriovenous Anastomoses in the Sympathetic Chain

Lattes

PLATE 45

FIG. 9. Photomicrograph showing an arteriole and smaller but similar vessels all surrounded by prominent, clear, smooth muscle cells. Between them are large, irregular, clear elements with vesicular nuclei and occasional eosinophilic cytoplasmic granules, apparently derived from the adventitia of the same vessels, and suggesting a pericytic nature. This probably represents an early stage in the histogenesis of one of the vascular glomeruli. Hematoxylin and eosin stain. $\times 450$.

FIG. 10. Photomicrograph of a sympathetic ganglion showing a capillary blood vessel (A) emptying through a funnel-shaped channel into a large venous sinusoid (B). The other capillary blood vessel (C) running in a transverse direction is not connected with the sinusoidal space. Hematoxylin and eosin stain. $\times 570$.



Lattes

Arteriövenous Anastomoses in the Sympathetic Chain

LESIONS IN ELASTIC ARTERIES ASSOCIATED WITH HYPERTENSION *

C. T. ASHWORTH, M.D., and D. M. HAYNES, M.D.

(From the Departments of Pathology of the Southwestern Medical College and Parkland Hospital, Dallas, Texas)

It is a well established fact that morphologic alterations in the vascular system occur in hypertension, and extensive investigations have been carried out on this basis. One of the most constant of these alterations is intimal hyalinization and medial hypertrophy in the arterioles. This was described by George Johnson¹ in 1852, and designated arteriolar sclerosis. The description of this lesion by Gull and Sutton² established its morphologic characteristics, and the observations of Jores,³ Bell and Clawson,⁴ Fishberg,⁵ and Moritz and Oldt⁶ have added to the knowledge of this subject. The causal relationship between arteriolar sclerosis and hypertension, however, remains somewhat uncertain. The demonstration of the absence of arteriolar sclerosis in early cases of essential hypertension by Castleman and Smithwick⁷ has been interpreted as indicating that arteriolar sclerosis is the result, rather than the cause, of hypertension.

Certain regressive and progressive changes occurring in the muscular arteries have been described from time to time in connection with the hypertensive state. Medial calcification, belonging to this group, was first observed by Virchow.⁸ Mönckeberg,⁹ however, gave the classical description and interpretation of this lesion, and it is now often referred to as Mönckeberg's sclerosis. Medial calcification in the arteries of the lower extremities apparently begins during childhood and increases in incidence and in severity with age. Although, as Bell¹⁰ pointed out, hydrostatic pressure appears to contribute to its development, since the lesion is much more marked in the lower than in the upper extremities, Dietrich¹¹ found no definite correlation existing between medial calcification and hypertension.

In the muscular arteries, Marchand¹² and Dietrich¹³ found the medial coat to have undergone hypertrophy in hypertensive subjects. In a more recent study, however, Zacharjewska¹⁴ was unable to substantiate this observation in so far as the renal arteries are concerned.

Ectasia of the muscular, as well as elastic, arteries has been shown to occur in the senile period of life, and often in the absence of hypertension. Dietrich,¹³ however, has shown that chronic hypertension is also a very important factor in the production of dilatation of arteries. He noted that in young and middle-aged persons, ectasia of the muscu-

* Received for publication, April 2, 1947.

lar arteries occurred only in the presence of hypertension. Furthermore, the dilatation was more marked in senile patients if hypertension was present.

A large number of observations have been made concerning the alterations in the elastic arteries associated with hypertension. Aortic arteriosclerosis as well as that of coronary and cerebral arteries, is regularly considered to be more severe and more common in the presence of prolonged hypertension. The medionecrosis aortae idiopathica of Erdheim occurs usually in patients who are hypertensive, and, by virtue of this fact, dissecting aneurysm of the aorta is commonly associated with the pre-existing hypertension. In a careful microscopic study of the aortas in 210 routine autopsy cases, Rottino¹⁵ noted the frequent occurrence of simple muscle loss of the media, degeneration of the elastic lamellae, and foci of cystic mucoid degeneration of the media. These alterations were slightly more common and more severe in the hypertensive than in the nonhypertensive subjects of this study.

An alteration of the elastic arteries has been noted in three hypertensive patients. Apparently this process has not been previously described.

REPORT OF CASES

Case 1

A colored male, 55 years old, was admitted to Parkland Hospital with motor aphasia and hypertension. The extremities were cold, and light palpation over the legs was painful. Multiple soft subcutaneous nodules were distributed over the body. This patient had been in the hospital on two previous occasions: 5 years and 1 year, respectively, prior to the final admission. At the time of the first admission he had experienced a sudden onset of pain and numbness in the left arm, and axillary, radial, and ulnar pulsations were absent on that side. A scalenotomy was performed without remarkable improvement. One of the tumor masses was removed and diagnosed as "neurofibroma with myxomatous degeneration." Four years later, he was admitted complaining of headache, dizziness, and nausea. The blood pressure was 150/100 mm. Hg. The blood urea was 64 mg. per cent. The Kline test was negative and the blood urea was 16 mg. per cent. After 2 weeks of hospitalization without specific therapy of any sort, the patient's general condition had greatly improved. The clinical diagnosis was hypertensive encephalopathy. During his final admission, the patient suddenly became unconscious and exhibited hemiplegia. He expired quietly on the tenth hospital day.

At autopsy, there were numerous small subcutaneous neurofibromas over the entire body, but they were most numerous on the trunk. Focal areas of hyperpigmentation were present in the skin of the back. There were multiple, large, partially organized thrombi in the thoracic and abdominal aorta; the superior portion of the ascending thoracic aorta showed almost complete occlusion, as did the first artery arising from the arch, an arterial stem which gave rise to the two common carotid arteries. The second branch from the arch was the left subclavian artery, while the third and distal branch extended posteriorly, cours-

ing behind the esophagus, then to the right, becoming the right subclavian artery. There was a mural thrombus of the abdominal aorta, with extension into the superior mesenteric artery, and complete occlusion of the distal 8 cm. of the aorta. Thrombi also were present in both iliac and femoral arteries, in the left ventricle, in the branches of the small pulmonary artery, and in the splenic artery and vein. The left suprarenal medulla was the site of a tumor mass measuring 5.5 by 4.4 by 3.2 cm. Sections from this lesion showed it to be a paraganglioma. There was an old area of encephalomalacia in the left temporoparietal region, involving the area of the word-memory center. There were bilateral cervical ribs, and moderate arteriosclerosis of the aorta and coronary arteries.

Case 2

The patient was a white male, 36 years old, who had had known diabetes mellitus for 5 years, and had been admitted to the hospital many times for treatment of various infections and once with edema of the leg and hypertension. He was admitted to Parkland Hospital complaining of recurrent episodes of nausea and vomiting of 17 days' duration, and generalized edema for the same period. Examination revealed a blood pressure of 180/112, generalized edema, scarring and fresh hemorrhages in both optic fundi, and limitation of vision to light perception. Hemoglobin was 12.2 gm.; red blood cell count, 3,110,000; white blood cell count, 12,600 per cmm., with a normal differential count. The urinary findings were: specific gravity, 1.011; albumin, 3 plus; sugar, 1 plus; many red blood cells, few white blood cells, and occasional casts. Chemical studies of the blood showed: sugar, 200 mg. per cent; albumin, 2.4 gm. per cent; globulin, 2.8 gm. per cent; urea, 184 mg. per cent; cholesterol, 429 mg. per cent. The Kline test was negative. For the 22 days of his hospitalization, the patient was nauseated, continued to be edematous, and eventually became alkalotic, with CO₂-combining power of 81.4 volumes per cent. Tetany was treated by calcium gluconate injections. He became more edematous, and progressively drowsier, finally lapsing into coma and expiring. The clinical diagnosis was Kimmelstiel-Wilson disease.

At autopsy 5 hours after death there was marked pitting edema of feet and ankles, right leg, and abdominal and chest walls. The peritoneal cavity contained 6,000 cc. of a thin fluid, and there were 100 cc. of colorless fluid in the pericardial sac. The heart was not enlarged. Moderate arteriosclerosis of the aorta, aortic valve, and anterior mitral leaflets was noted, and there was marked coronary arteriosclerosis with narrowing of the lumina. The left ventricle showed focal areas of fibrosis. The lungs were the seat of acute passive hyperemia and edema, and showed focal patches of bronchopneumonia. The kidneys were pale, and demonstrated a smooth surface, with prominent blood vessel markings; cut section revealed a markedly pale gray structure, the cortex measuring 9 mm. in thickness and showing indistinct cortical markings. The glomeruli could easily be seen as very pale yellowish, opaque bodies. The pyramids were indistinct, and considerably enlarged. Microscopic examination showed marked diffuse fibrosis and

hyaline replacement of glomeruli, alternating with areas of structurally preserved renal tissue with dilated tubules. The interstitial tissue showed focal and diffuse lymphocytic infiltration, and there was slight to moderate arteriosclerosis and arteriolar sclerosis. The diagnosis was chronic and subacute glomerulonephritis. The duodenum showed acute mucosal erosion and small hemorrhages. There was an area of marked liquefaction in the superior portion of the left lentiform nucleus; the encephalomalacic area extended into the surrounding white substance of the cerebrum, and the internal capsule was edematous.

The orifice of the left common carotid artery was completely occluded by a moderately friable, adherent, dark reddish gray thrombus showing partial organization. This thrombus extended to the bifurcation of the common carotid artery.

Case 3

A white male, 60 years old, entered Parkland Hospital complaining of asthma, orthopnea, and productive cough of many years' duration. He gave a history of known hypertension for 2 years, and had had attacks of paroxysmal nocturnal dyspnea for 1 year. There had been blurring of vision and severe frontal headache for 2 months prior to admission, and for 2 weeks he had been lethargic and oliguric, and suffered from anorexia. On examination he was found to be emaciated, lethargic, and dyspneic. Temperature was 98.6° F.; blood pressure, 210/126. There was edema of the anterior chest and abdomen, along the posterior axillary folds, and over the sacrum. Dullness was elicited over the right lower lobe. The heart was not enlarged, and auscultation revealed premature auricular beats. The hemoglobin was 10 gm., and the white blood cell count was 15,400 with a shift to the left in the Schilling hemogram. The specific gravity of the urine was 1.012 and the albumin was 4 plus. Chemical studies of the blood showed: urea, 105 mg. per cent; total serum proteins, 7.3 gm. per cent; albumin, 3.3 gm. per cent; globulin, 4.0 gm. per cent; CO₂-combining power, 53.1 volumes per cent. Venous pressure was 65 mm. of water, and the circulation time was 23 seconds with decholin. The Kline test was negative. The patient received digitalis and aminophylline, and seemed to be improving, when he quietly expired on the eighth hospital day. The clinical diagnoses were "chronic glomerulonephritis, bronchial asthma, pulmonary emphysema, and generalized arteriosclerosis."

At autopsy, the peritoneal cavity contained 5,500 cc. of colorless fluid. The aorta showed ulcerated and hemorrhagic atherosclerotic plaques. There was moderate left ventricular enlargement of the heart, the left wall measuring 2 cm. in thickness. There was marked coronary atherosclerosis. The lungs showed acute passive hyperemia, slight edema, and chronic fibrous pleuritis on the right. The kidneys were pale and presented a finely granular surface with many uniformly distributed areas of indentation. The cortical markings were not prominent. Microscopic study showed a marked degree of arteriolar nephrosclerosis.

In the gross examination of the elastic arteries of these three cases, thrombosis of the aorta and other arteries in case 1 and of the left

common carotid artery of case 2 is the outstanding observation. Atherosclerosis was evident in some degree in each case; however, no further alterations were noted. A composite description of the microscopic changes in the elastic arteries of these three cases follows.

HISTOLOGIC OBSERVATIONS ON INVOLVED ARTERIES

The principal alteration in the elastic arteries was in the medial coat. The outer musculo-elastic layers appeared to be partially collapsed, so that individual elastic lamellae lay close together and were for the most part intact. Some elastic fibers were broken into small, separate, refractile particles and were undergoing disintegration. This outer portion of the medial coat was the seat of marked increase in cellularity, due to proliferation and exudation. Elongated, fixed cells resembling fibroblasts were the proliferating constituents and these were regularly arranged parallel with the lamellae of the medial coat. The nuclei of these cells were hypertrophied and pale. Many new capillaries also were present in the area, extending in from the adjacent adventitia. Many of these vessels were represented by small, solid cords of endothelial cells without lumina while others possessed small lumina. The endothelial cells, like the fibroblasts, had swollen, hypochromatic or vesicular nuclei. Besides the young capillary units, there also were numerous thin-walled vessels, predominantly veins, which extended into the media from the adventitia. Some of these were arranged at right angles, while others extended circularly around the vessel. The degree of proliferation and vascularization was marked in cases 1 and 2, while in case 3 it was slight.

The exudative constituent of the medial lesion consisted of moderate numbers of lymphocytes which were diffusely scattered throughout the outer medial coat, being most numerous in the regions of capillaries and where proliferative changes were most pronounced. Occasional polymorphonuclear cells and large mononuclear wandering cells were present also. In case 3, in which there was marked fragmentation of the musculo-elastic layer and small foci of coagulative necrosis in the outermost portion of the media, polymorphonuclear cells were the main exudative component.

In the adventitia there was a slight to moderate infiltration of lymphocytes, which were primarily collected in the region of blood vessels. These cellular infiltrates differed in appearance from those seen in syphilitic aortitis in their failure to form complete and intimate perivascular collars. The infiltration did not involve the vasa vasorum, and these vessels were not the seat of endarteritis. In the portion of the adventitia adjacent to the media, the collagenous bundles were

thickened and there was fibrous tissue proliferation, leading to slight cicatrization. Collagen bundles near the media were found to be markedly fragmented and occasionally the seat of early coagulative or fibrinoid necrosis. Capillaries with swollen endothelial cells were noted in this portion of the adventitia.

The vasa vasorum were found to have thickened walls so that the lumen/wall ratio was markedly reduced. There was a slight degree of hyaline deposit in the intima, but the most striking change was thickening of the muscle coat of the vasa vasorum. The muscle fibers of the media were distinctly increased in number, so that several layers of muscle cells had developed in these vessels. The individual muscle fibers were hypertrophied and their nuclei swollen.

In each of the cases reported there was atherosclerosis, involving the intima. In cases 1 and 2 atherosclerosis was slight or moderate. In case 3 it was marked, but in this instance the lesions described above were found in portions of the aorta where atherosclerosis was slight.

HISTOLOGIC STUDY OF AORTA AND OTHER ELASTIC ARTERIES IN HYPERTENSIVE AND NONHYPERTENSIVE PATIENTS

Microscopic study of the aorta, and in many cases also of the innominate, left common carotid, and left subclavian arteries, was made in 40 patients who had hypertension. Thirty-one of this group had essential hypertension, 5 had pyelonephritis, 3 had glomerulonephritis, and one had hypertension due to a paraganglioma of the adrenal.

Atherosclerosis was present in at least a mild degree in every case, and in general it was considerably more marked in the hypertensive group than in the control group of nonhypertensive patients. In 15 patients atherosclerosis of the elastic arteries was the only alteration present on gross and microscopic examination. Two patients had syphilitic aortitis. The remaining 23 cases revealed some degree of histologic alteration of the aorta which was considered to be distinct from the effects of atherosclerosis. The histologic changes were marked in 6 of these, moderate in 11, and slight in 6. Two patients in this group had arteriosclerotic aneurysms of the abdominal aorta.

The histologic changes referred to were located in the media and adventitia and also involved the vasa vasorum. The most common alteration consisted of small collections of lymphocytes in the adventitia. Except in those cases in which syphilitic aortitis was present, no fibrous proliferation was encountered. In the media, increase in number and in prominence of capillaries and other small vessels were very common observations. The endothelial cells lining these small vessels often were swollen. Small numbers of lymphocytes and polymorphonu-

clear leukocytes frequently were seen around these vessels in the media. Occasionally, small foci of degeneration or necrosis were noted in the outer portion of the media, associated with disruption of the medial elastic and muscular lamellae. Less common observations relating to changes in the media were atrophy of the musculo-elastic lamellae with reduction in thickness of the layer, areas of limited fibrous replacement, and mucoid degeneration.

In the vasa vasorum, hypertrophy of the muscle coat and, occasionally, subendothelial hyaline deposition were commonly noted. The incidence of this arteriolar sclerosis was 35 per cent.

It will be seen that these alterations occurring in the aortas of a series of hypertensive patients are similar, except for their milder form, to those described in the three reported cases.

In a group of 50 control cases without hypertension, microscopic examination of the elastic arteries failed to reveal alterations approaching those which have just been described. Atherosclerosis of marked degree was present in numerous cases and occasionally in such cases, small collections of lymphoid cells were noted in the adventitia. Hypertrophy and hyaline deposition were not found in the vasa vasorum in any case. Atrophy of the media associated with marked atherosclerosis was noted occasionally. Otherwise medial changes were not encountered.

Analysis of the clinical and pathologic data in the hypertensive series showed that there was no correlation between the incidence or the severity of the changes in the aorta, and the age or sex of the patients, uremia, the level of blood pressure, the etiologic mechanism of the hypertension, or serologic and pathologic evidence of syphilis. ~

On the other hand, there was noted a certain degree of correlation between the severity of the lesions of the medial and adventitial coats of the aorta and the severity of the atherosclerosis. The degree of correlation is not great, and there were several cases of marked medial and adventitial alterations with only slight atherosclerosis. In a considerable number of cases of marked atherosclerosis medial and adventitial changes were slight or absent.

COMMENT ON PATHOGENESIS AND SIGNIFICANCE OF ARTERIAL LESIONS

The lesions described here bear some similarity to periarteritis nodosa. Especially since recent studies by Cromartie,¹⁰ and Loomis¹⁷ have demonstrated the occurrence of periarteritis in rats which had been rendered hypertensive, the possibility of so designating the lesions described here must be considered. The distribution of the arterial lesions in the cases reported here is not that of periarteritis as seen

clinically, nor is it that of the type of periarteritis observed in hypertensive rats. In Loomis' study, the mesenteric arteries were primarily involved, although lesions in the elastic arteries were recorded and described. The principal involvement of the media, the presence of degenerative changes in the elastic fibers, the muscular hypertrophy of vasa vasorum, and the absence of eosinophils in the lesions as described, indicate that fundamental differences from periarteritis nodosa exist. None of the three patients reported here had received sulfonamide drugs, although periarteritis certainly occurs in the absence of such medication.

From the morphologic standpoint it appears relatively certain that this arterial lesion is not due to syphilis. The absence of typical perivascular lymphoid collars in the adventitia, of endarteritis of the vasa vasorum, of characteristic meso-arteritis, and of gummatous necrosis of the media are the main points of difference between the arterial lesion described and that of syphilis. Serologic tests for syphilis were negative in the reported cases and no other visceral lesions of syphilis were found. Likewise, the occurrence of less severe aortic lesions of the same type were noted in the series of hypertensive cases in which no correlation with serologic or pathologic evidence of syphilis could be demonstrated.

Observations on medial changes of the elastic arteries in hypertension must take into account the lesion described by Erdheim,¹⁸ named by him "medionecrosis aortae idiopathica," and since studied by Moritz¹⁹ and by Rottino.²⁰ This lesion, as pointed out by various observers, is a cystic necrosis developing in the central portion of the media, and absence of proliferative and exudative reaction is characteristic of the process. In this respect there is a fundamental difference between this type of medial necrosis and the lesion reported and described here.

There have been several reports^{21,22} of experimental studies which indicated the occurrence of changes in arteries due to the administration of adrenalin and other vasoconstrictor agents. These authors have reported degenerative changes, necrosis, and secondary inflammatory reaction in the media and adventitia of the aorta and of other vessels throughout the body. The changes described are similar to those reported here in human subjects with hypertension. In view of the muscular hypertrophy of the vasa vasorum of the vessels involved in our cases, and in view of the vasoconstrictive state which is understood to accompany the hypertensive process, we may assume the pathogenesis of the lesions described here to originate with constriction of the vasa vasorum. This vasoconstriction would necessarily lead to anoxia

of the arterial wall, which, if severe enough, would produce degenerative changes, necrosis, and secondary inflammatory reaction in the vessel wall. In case 1; as reported, the mechanism of constriction of the vasa vasorum is apparently the excessive output of adrenalin by the paraganglioma. In the other cases, one of hypertension associated with chronic glomerulonephritis and the other of essential hypertension, the constriction of the vasa vasorum might be attributed to the general vasoconstrictive mechanism in these cases. The degenerative and inflammatory arterial lesions would be expected to occur more frequently and in more severe form in those cases in which the hypertension is of a severe grade.

One may consider what the primary event is when the medial lesions here described are found accompanying atherosclerosis. In syphilis of the aorta it is well known that the changes in the outer layers of the aorta predispose to atherosclerosis. Although it is quite certain that intimal lipid deposition and hyalinization may occur in the presence of an otherwise entirely normal vessel wall, nevertheless it seems likely that the intimal deposits are hastened and exaggerated by medial disease. This explanation might account in part for the exaggerated atherosclerosis in the aortas of patients with hypertension, since degenerative and inflammatory medial lesions seem to occur in this group. However, increased pressure within the lumina of the elastic arteries appears to be a factor of great importance in the development of atherosclerosis in hypertensive patients.

The occurrence of marked thrombosis of the large arteries which was observed in two of the reported cases is of considerable importance. In case 1, this phenomenon was at least contributory to the production of death, if not the main factor therein. Atherosclerosis, which was present in mild to moderate degree in these cases, cannot be considered the causative factor of the thrombosis since very severe and "ulcerative" atherosclerosis of the aorta produces only very small thrombi over the ulcerated plaques. Furthermore, the atherosclerosis in the two cases with thrombosis was not extreme, nor was it ulcerative. We are led to believe that the same vasoconstrictive anoxia, which is thought to account for the medial and adventitial injury, likewise produced an extensive intimal endothelial injury followed by thrombosis.

It is tenable that the vasoconstrictive anoxia developing in this manner and present over a long period, produces the type of intimal injury which may be followed by atherosclerosis, according to the anoxic theory of atherosclerosis recently detailed by Hueper.²³ On the basis of this hypothesis, it is possible to account for the development of both medial lesions and atherosclerosis in hypertensive disease, and to ac-

count for the correlation in severity of these two apparently separate lesions that has been shown to exist in this report.

SUMMARY

Three cases are reported in which unusual lesions were noted in the aorta and large elastic arteries accompanying hypertension. In two cases thrombi were present in the involved arteries. This alteration of the elastic arteries involved the media most strikingly. In its outer portion there were degeneration, necrosis, and collapse of the musculo-elastic lamellae, accompanied by an exudation of lymphocytes and other inflammatory cells, and by proliferation of fibroblasts and capillary endothelial cells. In the adventitial layer there were perivascular lymphocytic infiltration, and muscular hypertrophy and hyperplasia with subendothelial hyaline deposition of the arteriae vasorum.

In a review of 40 hypertensive cases, similar but much less marked alterations of the aorta and/or other large elastic arteries were noted in 23.

On the basis of histologic findings, serologic tests, and other accompanying pathologic and clinical data, it is considered that the lesions of elastic arteries described are separate and distinct from atherosclerosis, syphilitic arteritis, periarteritis nodosa, and medionecrosis of the aorta. It is suggested that constriction of the vasa vasorum associated with the hypertensive state might lead to ischemia with injury of medial and adventitial layers resulting in the degenerative, inflammatory, and proliferative alterations noted, and that thrombosis might be explained on the basis of ischemic intimal injury.

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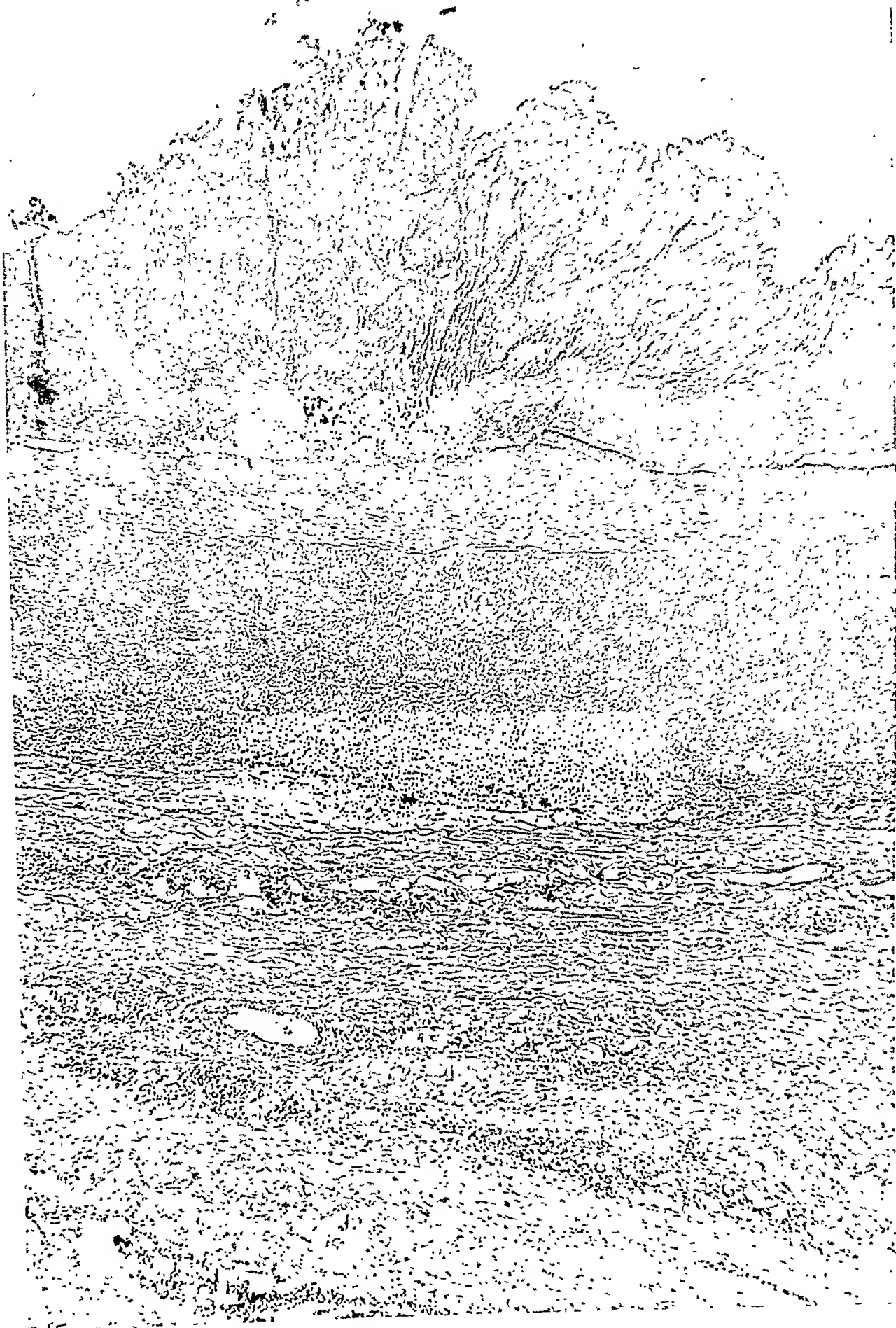
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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 46

FIG. 1. Photomicrograph of the aorta in case 1. A portion of the thrombus is adherent to the intima. Slight atherosclerosis is present. The outer medial coat shows marked exudative and degenerative changes. Scarring and lymphoid infiltration are present in the adventitia. Hematoxylin and eosin stain. $\times 130$.



Lesions in Elastic Arteries

Ashworth and Haynes

PLATE 47

FIG. 2. Photomicrograph of the left common carotid artery in case 2. An organized thrombus occupies the lumen. The outer medial coat and adventitia are infiltrated with lymphocytes. Of note are the degeneration and necrosis of the medial musculo-elastic lamellae. The arteriae vasorum in the adventitia reveal medial hypertrophy. Hematoxylin and eosin stain. $\times 175$.

2



ALTERATIONS OF CEREBRAL CAPILLARIES IN THE EARLY STAGE OF ARTERIAL HYPERTENSION *

I. MARK SCHEINKER, M.D.

(From the Laboratory of Neuropathology, Cincinnati General Hospital, and the University of Cincinnati, College of Medicine, Cincinnati, Ohio)

The paramount significance of arterial hypertension in the production of cerebral tissue alterations has been emphasized repeatedly in recent studies.¹⁻⁴ The object of this paper is to analyze the *earliest* stage of cerebral vascular changes in cases of arterial hypertension, with special emphasis upon those seen in the cerebral capillaries. This study is based upon 6 fatal cases of arterial hypertension in which death occurred from 1 to 2 years after the onset of the disease. No attempt will be made to describe the findings in each case. Two illustrative cases, however, are presented in detail.

REPORT OF CASES

CASE I

A white male, 50 years of age, was admitted to the hospital with a history of arterial hypertension of 1 year's duration. He had been in good health until 2 weeks prior to admission, at which time he noticed severe frontal and occipital headache, malaise, and shortness of breath. During the last few days, the patient had had several bouts of paroxysmal nocturnal dyspnea. His wife stated that, within the past 2 weeks, the patient had shown occasional signs of confusion, disorientation, and forgetfulness.

Examination. Upon admission the patient was extremely restless and confused. His temperature was 100° F.; pulse, 94; respirations, 20; blood pressure, 230/140 mm. Hg. There was a suggestion of bilateral exophthalmus. The pupils were 4 mm. wide, and reacted normally to light and in accommodation. Examination of the fundi disclosed bilateral papilledema and a few hemorrhages. The neurologic findings were normal.

Course. The initial state of confusion progressed rapidly through delirium to stupor and coma. The patient finally developed generalized convulsions, and died on the ninth hospital day.

Laboratory Data. A lumbar puncture performed on the third hospital day yielded clear, colorless cerebrospinal fluid under pressure of 600 mm. of water; it contained no cells; the total protein content was 138 mg. per 100 cc., and the Wassermann reaction was negative. A spinal tap performed on the seventh hospital day yielded xanthochromic fluid under pressure of 210 mm. of water; it contained no cells. The urine was grossly bloody and contained granular casts and albumin. The blood urea nitrogen on the second day after admission was 130 mg. per 100 cc. and subsequently (3 days before death) rose to 210 mg. per 100 cc.

Necropsy Findings

The pathologic alterations, exclusive of those found in the brain, were summarized as bilateral confluent lobular pneumonia; cardiac

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hypertrophy and dilatation; arteriolonephrosclerosis; right pleural and pericardial fibrosis; and petechial hemorrhages in the pleura, kidneys, pelvis, and urethra.

Examination of the nervous system was limited to the brain, which weighed 1400 gm. and showed a generalized increase in bulk. The gyri were flattened and the sulci narrowed. There was a slight increase in subarachnoid fluid in the parietal region near the midline. The pons, medulla, and cerebellum were not remarkable. There was a well defined cerebellar pressure cone. The blood vessels of the circle of Willis were normal in distribution and showed changes characteristic of arteriosclerosis.

Coronal sections through both hemispheres revealed a diffuse swelling of the white matter of the left hemisphere, with consequent compression of the left lateral ventricle. In the right hemisphere there was a massive hemorrhage in the vicinity of the island of Reil and lateral to the internal capsule. Sections of the brain stem disclosed a small ball hemorrhage on the left side of the pons. In addition there was a diffuse swelling of the midbrain, with loss of demarcation between gray and white matter. Sections of the medulla and cerebellum revealed no gross abnormalities.

Microscopic Findings

Histologic examination disclosed abnormalities of two principal types: (1) vascular alterations, and (2) changes in the nervous tissue.

The chief histologic alterations of the vascular system are shown in Figures 1 and 2. The vascular changes were confined essentially to the capillaries. These exhibited a uniform pathologic process consisting of a combination of extreme cellularity and degenerative alterations of the vessel walls. The increased cellularity of the capillaries was the most striking and the most widespread vascular alteration. It was caused chiefly by an extreme degree of fibroblastic proliferation of the adventitial membrane and a pronounced hyperplasia of the lining endothelium. In addition there was a pericapillary accumulation of glial nuclei, resulting from the marked glial hyperplasia and hypertrophy of the surrounding nervous tissue. Only occasionally were there seen a small number of macrophages and a few lymphocytes.

The degenerative alterations consisted of homogenization and thickening of the vessel walls with resultant narrowing or complete obliteration of the vascular lumina. In many instances the entire capillary was converted into a structureless, homogeneously stained, hyalinized, solid nodule composed of a central homogeneous mass surrounded by a ring of proliferated fibroblasts and glial cells (Fig. 3). The central portion obviously represented the remnants of the completely degenerated and hyalinized capillary, the lumen of which was obliterated.

The arterioles exhibited only a moderate degree of thickening and hyalinization of their walls. Changes characteristic of advanced hypertensive arteriolopathy were seen only occasionally.

More conspicuous were the changes of the cerebral veins. In addition to the massive hemorrhage involving the right basal ganglia, there were a few so-called ball hemorrhages in the form of small, sharply demarcated hemorrhagic foci measuring from 5 to 50 mm. in diameter. They were seen in areas quite remote from the massive hemorrhage, and consisted of one or several central veins surrounded by large masses of extravasated blood. The central blood vessels were mostly medium-sized, extremely congested veins; their lumina were distended, and far advanced degenerative changes had occurred in their walls.

The degree of degeneration of some of the veins is illustrated in Figure 4. As here shown, the vascular wall had undergone almost complete disintegration and necrosis. The normal appearance of the division into three coats was completely lost. The entire vessel wall was transformed into a structureless, homogeneous substance devoid of either muscular or elastic elements. Its outer margin was bounded by a thin connective tissue membrane with a small number of nuclei which were barely visible. In some instances, the perivascular space of the vein was distended and harbored red blood cells and macrophages.

Changes of the nervous tissue included both diffuse alterations of the parenchyma and vascular alterations characteristic of cerebral swelling.⁵ Figure 5 illustrates the characteristic spongy appearance of the central white matter caused by the tremendous distention of the pericellular and perivascular spaces and by numerous large, oval spaces filled with serous fluid. The latter contained neither cellular elements nor stainable substance. Numerous nerve fibers and myelin sheaths displayed various stages of degeneration, such as irregularity of contour, beading, or complete loss of stainability.

The general vascular alterations consisted of congestion, stasis, and morphologic signs of vasoparalysis of the smaller veins and capillaries. In many smaller blood vessels there were various degrees of degeneration of the walls, associated with increased permeability for serous fluid which had transuded into the distended perivascular spaces. These changes were most pronounced within the swollen portions of the white matter.

CASE 2

A white female, 41 years of age, was admitted to the hospital with a history of hypertension of approximately 2 years' duration. She was first admitted in November, 1945, because of attacks of asthma associated with purulent bronchitis and fever. On routine examination hypertension of 205/140 mm. Hg was found. There had been no symptoms related to high blood pressure except occasional headaches.

Physical examination disclosed the following pertinent findings: The optic disks were mildly congested, but there was no definite papilledema. The retinal arterioles showed attenuation and areas of localized spasm, with compression at venous crossings; there were multiple cotton-wool patches of exudate and perivascular flame-shaped hemorrhages in both eyes. The neurologic examination disclosed normal findings.

Laboratory Findings. Concentration tests of urine showed a variation from 1.006 to 1.016 in specific gravity. Repeated urinalyses for sugar and albumin were negative; only on one occasion was 1 plus albumin found. The sediment contained only a few white blood cells and was otherwise negative. The blood urea nitrogen was 24, 23, and 17 mg. per 100 cc. Other studies, including phenolsulfonphthalein test and pyclography, were normal. Electrocardiographic examination of the heart yielded normal findings.

Second Hospital Admission. On February 18, 1946, the patient was admitted for the second time. For the past 3 months she had been kept on a low sodium diet. Her chief complaints were orthopnea, dyspnea, and swelling of the ankles. Physical examination revealed signs of right-sided cardiac failure with hepatic congestion. The neck veins were distended; the venous pressure was 24 cm. of water. The hepato-jugular reflex was positive.

Clinical Course. During the second hospital week the patient began to complain of visual disturbances. Examination of the fundi disclosed papilledema, more pronounced in the right eye than in the left. The retinal veins were distended; no fresh hemorrhages were seen.

The patient frequently complained of nocturnal respiratory distress. During her hospital stay she had several attacks of asthma. The blood pressure varied between 194/130 and 210/140.

On March 21, 1946, a left Smithwick procedure was performed. The patient tolerated the operation quite well, and the postoperative course was uneventful. Her blood pressure remained essentially at the same level, varying between 210/130 and 190/140. Following the operation she complained of complete loss of vision in the right eye. Ophthalmoscopic examination disclosed extensive edema of the retina and of the optic disk on the right. On April 1, 1946, a right Smithwick procedure was performed. At the end of the operation, as the skin was being closed, the patient ceased to breathe and could not be revived.

Necropsy Findings

The pathologic changes, exclusive of those found in the brain, were summarized as acute catarrhal bronchitis with focal areas of pulmonary atelectasis; focal lobular pneumonia of the right lower lobe; right pneumothorax; accelerated arteriolonephrosclerosis; coronary, pulmonary, and aortic atherosclerosis; acute passive hyperemia of the viscera; mural thrombosis of the right atrial appendage; small focal areas of degeneration in the myocardium.

Examination of the nervous system was limited to the brain. On gross examination it appeared normal. The major blood vessels were thin-walled and of somewhat translucent appearance; small sclerotic plaques were seen only occasionally. Coronal sections through both hemispheres showed, except for a mild degree of congestion, normal findings. Sections through the midbrain revealed diffuse swelling of the tegmen of the pons, with almost complete obliteration of the lower

aqueduct and of the lumen of the fourth ventricle. In addition, there were a few small hemorrhages in the periaqueductal region.

Microscopic Findings

The most striking histologic findings were confined to the capillaries. These exhibited uniform pathologic lesions similar to those described in case 1. Figure 6 illustrates the increased cellularity of the capillary wall, caused chiefly by fibroblastic proliferation of the adventitial membrane and by hyperplasia and hypertrophy of the lining endothelium. The surrounding nervous tissue reacted, as a rule, with a focal area of pericapillary glial proliferation. In some instances, however, degenerative alterations of the capillaries were prevalent. These consisted of homogenization and thickening of the vessel wall, with resultant narrowing or complete obliteration of the vascular lumen (Fig. 7).

The arterioles were relatively slightly affected. Only occasionally were there changes characteristic of advanced hypertensive arteriopathy (Fig. 8).

Changes of the nervous tissue consisted of areas of rarefaction with early signs of necrosis. The nerve fibers and nerve cells showed loss of stainability and signs of ischemic degeneration. In addition there were a few perivascular hemorrhages in the periaqueductal region and in areas adjacent to the fourth ventricle.

Summary of Pathologic Findings

In previous contributions^{1,2} attention was drawn to vascular alterations in hypertensive encephalopathy. These histologic changes, which were regarded as typical, consisted of hyaline degeneration and fibrotic thickening of the vessel walls with narrowing or complete obliteration of the vascular lumina. These vascular changes, confined to arterioles and capillaries, are different from those found in arteriosclerosis and are to be interpreted as a special form of hypertensive arteriopathy. The alterations of the nervous parenchyma characterized by diffusely scattered small foci of old and recent softening were interpreted as secondary to the vascular lesions.

In addition to the arteriolar changes, two types of *venous* alterations were described recently^{3,4}: (a) reversible changes characterized by stasis, congestion, and distention of the vascular lumina; and (b) structural lesions of the vessel walls manifested by advanced signs of degeneration, necrosis, and/or an extreme degree of atrophy. Whereas the arteriolar changes were interpreted as significant for the diffuse focal areas of softening and gliosis of the nervous parenchyma typical

of hypertensive encephalopathy, the alterations of the cerebral veins were regarded as responsible for the origin and pathogenesis of the massive intracerebral hemorrhages so often encountered during the terminal phase of hypertensive brain disease.

In all cases in the present study the predominant pathologic alterations were confined to the capillaries. The vascular lesions were characterized by a combination of proliferative and degenerative changes of the capillary walls. The proliferative alterations were the result of fibroblastic proliferation of the adventitial membrane associated with pronounced hyperplasia and hypertrophy of the lining endothelium. The degenerative changes consisted of homogenization and thickening of the capillary wall with resultant narrowing or complete obliteration of the vascular lumen. In many instances the entire capillary was converted into a structureless, homogeneously stained, hyalinized solid nodule composed of a central homogeneous mass surrounded by a ring of proliferating glial cells and fibroblasts. The central portion obviously represented the remnants of the completely degenerated and hyalinized capillary, the lumen of which was obliterated.

In all cases under observation, death occurred from 1 to 2 years after onset of the clinical manifestations of the disease. It is concluded, therefore, that the afore-described capillary changes may be considered as the early stage of hypertensive brain disease.

Of special interest was the attempt to correlate the findings in the central nervous system with those in the kidneys. No direct parallelism could be established. Whereas in some cases the kidneys disclosed changes characteristic of accelerated nephrosclerosis, in others they displayed minimal changes or a moderate degree of an arteriosclerotic vascular process.

It is noteworthy that case 1 of the present study exemplifies a characteristic clinicopathologic syndrome of arterial hypertension recently described under the heading of "hypertensive cerebral swelling."⁶ The clinical picture is characterized by sudden onset and rapid progression of severe headache, drowsiness, confusion, restlessness, and delirium, accompanied by signs of increased intracranial pressure, such as elevation of spinal fluid pressure and papilledema. The underlying histopathologic findings consist in various stages of cerebral swelling.

Case 1 of this study revealed, in addition to cerebral swelling, a massive intracerebral hemorrhage and vascular alterations (venous and arteriolar changes) characteristic of hypertensive brain disease.

SUMMARY

Attention is directed to an early stage of cerebral vascular change in cases of arterial hypertension, in which the vascular alterations are

confined to the capillaries, and are characterized by a combination of proliferative and degenerative changes.

In all cases of the present study, death occurred from 1 to 2 years after onset of the clinical manifestations of the disease.

No direct parallelism between the cerebral vascular changes and those of the kidneys could be established.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 48

FIGS. 1 and 2. Case 1. Extreme cellularity and degenerative alterations of the cerebral capillaries. Hematoxylin and eosin stain. $\times 220$.

FIG. 3. Case 1. Structureless, hyalinized nodule composed of a central homogeneous mass surrounded by a ring of proliferated glial cells and fibroblasts. Hematoxylin and eosin stain. $\times 220$.

FIG. 4. Case 1. Disintegration and necrosis of a cerebral vein. Of note is the transformation of the vascular wall into a homogeneous substance devoid of either muscular or elastic elements. Hematoxylin and eosin stain. $\times 185$.

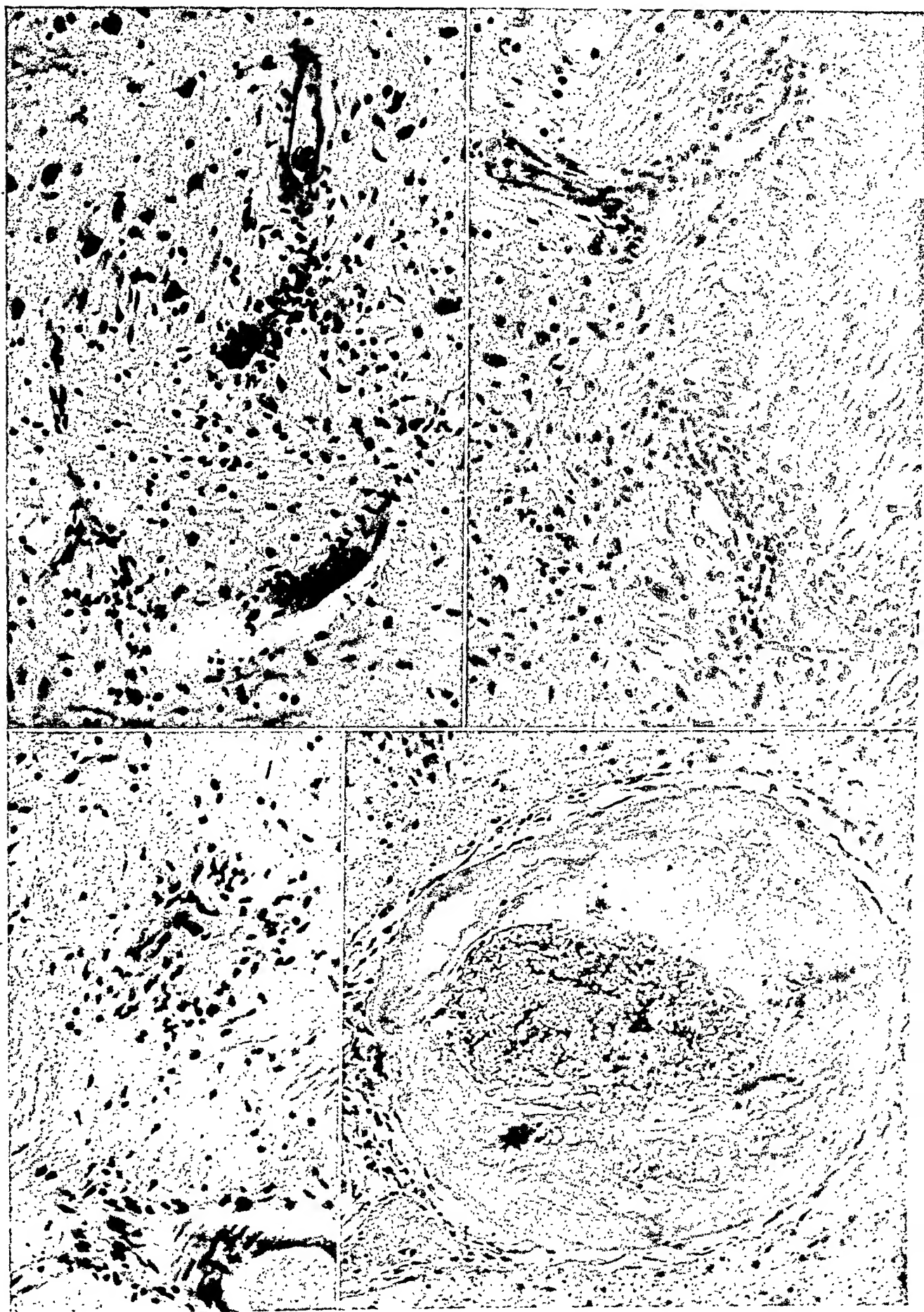
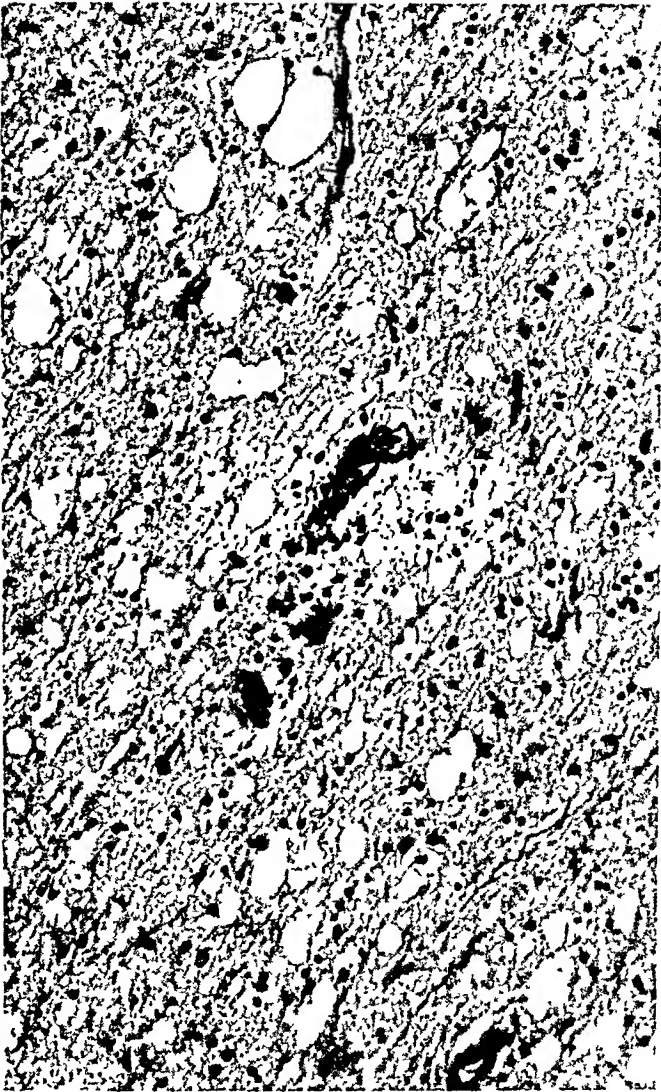


PLATE 49

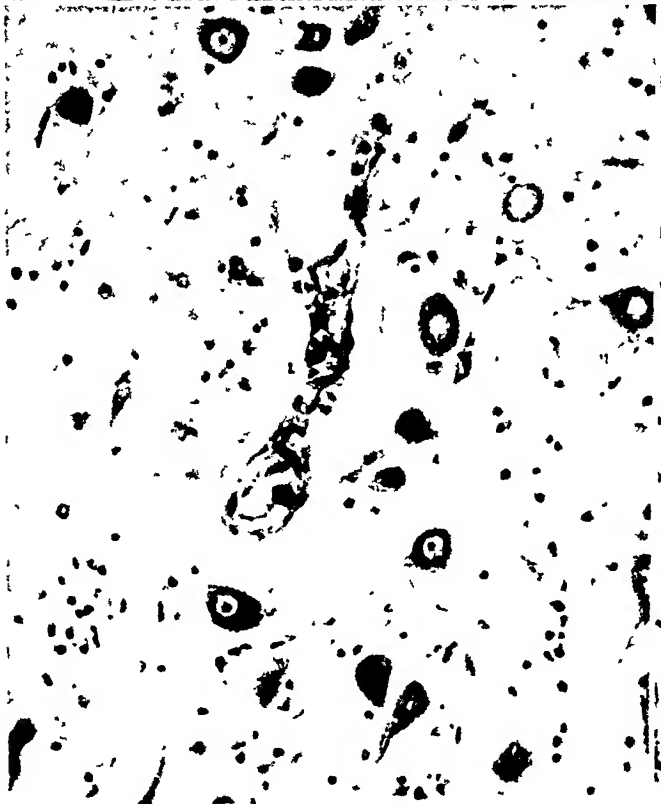
- FIG. 5. Case 1. *Spongy appearance of the white matter caused by numerous large oval spaces filled with serous fluid and by distention of the perivascular and pericellular spaces. Hematoxylin and eosin stain. $\times 185$.*
- FIG. 6. Case 2. *Increased cellularity of the cerebral capillaries caused by fibroblastic proliferation of the adventitial membrane and by hyperplasia and hypertrophy of the lining endothelium. Hematoxylin and eosin stain. $\times 220$.*
- FIG. 7. Case 2. *Homogenization and thickening of the capillary wall with resultant narrowing of the vascular lumen. Cresyl violet stain. $\times 220$.*
- FIG. 8. Case 2. *Arteriolar changes characteristic of hypertensive arteriolopathy. Of note is the extreme narrowing of the arteriolar lumen. Cresyl violet stain. $\times 220$.*



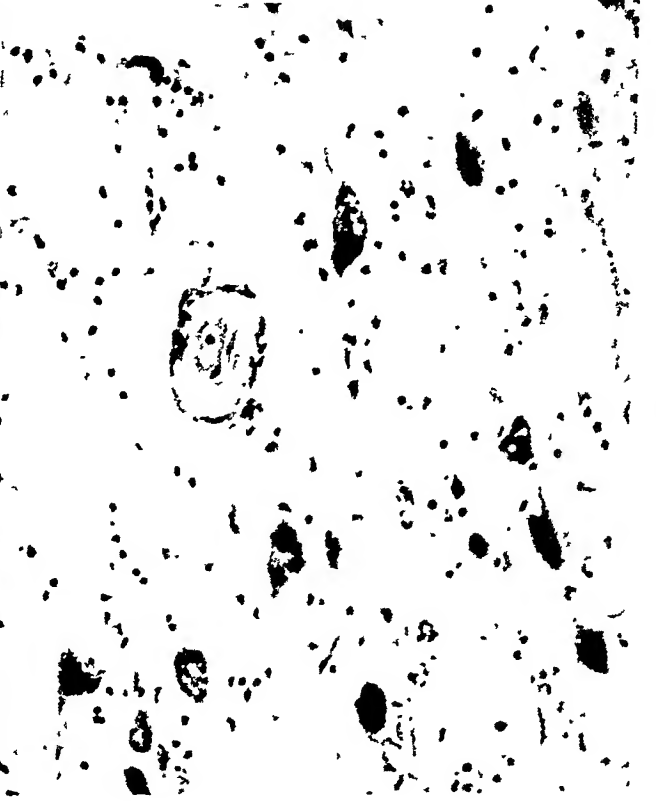
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COCCIDIOIDOMYCOSIS IN ANIMALS WITH REPORT OF A NEW CASE IN A DOG *

HILTON SMITH, D.V.M.

(From the Department of Veterinary Pathology, Division of Veterinary Medicine,
Iowa State College, Ames, Iowa)

The disease now known as coccidioidomycosis, or coccidioidal granuloma, was first encountered by Posada¹⁻³ in a human patient in Argentina in 1892; the same case was reported also by Wernicke.⁴ However, it has remained for the southwestern United States to supply the great majority of the several hundreds of cases in the world's literature. Most of these have come from southern California, particularly the San Joaquin Valley, but the disease has been reported (in human beings) also from the states of Washington, Arizona, New Mexico, Texas, Louisiana, Colorado, Kansas, Nebraska, Missouri, Illinois, Tennessee, South Carolina, and Pennsylvania, as well as from Alaska, Hawaii, and Italy.^{5,6} Very recently the disease has been reported from Army hospitals in a number of other states, but, owing to the constant movement of Army personnel, such cases were not necessarily indigenous to the areas from which they were reported.⁷

There is reason to believe that spores inhaled with dust in the desert countries of the Southwest are responsible for the more or less endemic status of the disease in these regions. It has been supposed that the causative fungus is capable of propagating on some native plant. On the other hand, Ashburn and Emmons⁸ trapped 105 rodents in the desert near San Carlos, Arizona, and found gross pulmonary lesions of coccidioidal granuloma in 9 of them (7 pocket mice, 1 kangaroo rat, 1 ground squirrel), suggesting that these creatures may act as "reservoirs of infection." Direct spread from diseased to healthy subjects does not occur, it is believed, either among man or animals.

Two forms of this infection are now recognized in man, one of them being a benign acute respiratory disorder sometimes known as valley fever. The other form is the classical coccidioidal granuloma, a chronic progressive affection characterized by widely disseminated foci of a specific granulation tissue around the causative organisms and having clinically and pathologically a close similarity to tuberculosis. It is only this granulomatous type that has been recognized in other animals than man.

The causative organism appears in the tissues of its host as a refractile, doubly contoured spherule, from 5 to 50 μ in diameter; the name *Coccidioides immitis* bespeaks its striking resemblance to a coccidial

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oocyst. In the process of multiplication the enlarged parent spherule, looked upon by systematists as an ascus, ^{9,10} becomes filled with small endospores (ascospores) which, at maturity, are released into the surrounding tissue. When transplanted to the surface of common bacteriologic media the organism readily develops an extensive mycelium, producing a white, fluffy colony. According to Dodge,⁹ there is no differentiation to form conidia.

Among animals, coccidioid granuloma has been found principally in cattle. Giltner ¹¹ reported the first case in 1918. It involved the bronchial and mediastinal lymph nodes of a bovine slaughtered at San Diego, California. He reproduced the disease in guinea-pigs and dogs, the inoculation of infectious material resulting in death within 2 months. About 70 additional cases in cattle have been brought to light in several reports by Stiles and co-workers ¹²⁻¹⁶ between 1933 and 1942. Beck, Traum, and Harrington ¹⁷ and Traum and Schalm ¹⁸ have reported approximately 20 cases. Their diagnoses were based upon demonstration of typical organisms in sections or cultures, or in both.

Findings in cattle may be summarized as follows: All cases have been from California, Arizona, or New Mexico with the exception of one from northern Colorado.¹² There were no observable clinical symptoms, the condition having been discovered in every instance in the course of routine veterinary inspection of carcasses slaughtered for food. Involvement was never very extensive, lesions being limited to bronchial and mediastinal lymph nodes, and, in a minority of instances, to small nodules in the lungs. The various descriptions of the lesions can be well represented by the words which Giltner ¹¹ applied to his case: "Large areas of suppuration or several smaller purulent foci, all of which are usually surrounded by considerable granulation tissue and a fibrous capsule. Upon incising an affected gland there may be squeezed out a thick yellowish and tenacious pus." Several authors have noted the similarity of an incised lesion to that found in actinomycosis.

Davis, Stiles, and McGregor¹⁴ described the histopathologic features as "granulomatous foci consisting of connective tissue, numerous blood-vessels, lymphocytes, plasma cells, mononuclear and polymorphonuclear leukocytes, a few eosinophiles, and many giant cells. The foci were surrounded by a connective-tissue capsule. Scattered throughout the inflammatory areas were many double-contoured spherical bodies, the majority of which had been taken up by giant cells. Scattered purulent areas containing spherical forms were seen within the granulation areas In some fields the inflammatory reaction resembled tubercle formations and only the presence of spherical bodies within the giant cells enabled a differentiation from a tuberculous process."

A striking feature of several of the bovine cases has been the presence of a "halo" of eosinophilic radiating "clubs" surrounding some of the *Coccidioides* spherules, entirely comparable to the "rosettes" of actinomycosis (as caused by *Actinomyces bovis*, also known as *Streptothrix israeli*) and actinobacillosis (caused by *Actinobacillus lignièresi*). This phenomenon has been described and illustrated by Davis, Stiles, and McGregor¹⁴ and by Traum and Schalm.¹⁸ Somewhat less pronounced manifestations of the same process have been seen in human tissues, as well as in experimentally inoculated guinea-pigs, with detailed studies by Moore.¹⁹ But the occurrence of this poorly understood reaction around *Coccidioides immitis* is far from usual in any species of host, and it appears especially well developed in the specimens from bovines.

Cattle have been inoculated artificially. When large doses of the organism are injected subcutaneously the usual result is a well encapsulated abscess at the point of inoculation and rather limited involvement of the lymph nodes draining that region.

One case has been reported in a sheep from California by Beck.²⁰ The disease has been found in a mountain gorilla (*Gorilla beringeri*) and in an American monkey (*Cebus hypoleucus*), both in a zoo in San Diego, California, as reported by McKenney, Traum, and Bonestell.²¹

Coccidioidal granuloma has been found twice in the dog. The first case was reported by Farness^{22,23} in 1940 and 1941 from Tucson, Arizona. The dog, a female Great Dane, 2 years old, was destroyed because of a gradually developing inability to use her hind legs, which was supposed to have been the result of severe rickets in early life. At necropsy, the lungs and, to a lesser extent, the liver, spleen, and kidneys, were found to contain numerous nodules resembling tubercles. Some of the nodules were described as necrotic. Microscopic sections made possible the diagnosis of coccidioidal granuloma, with demonstration of typical spherules. Since the brain and cord had not been examined, Farness pointed out the possibility that the inability to use the hind limbs might have been caused by a paralysis due to coccidioidal infection of the cord or its meninges, a type of involvement that is not rare among human cases.

The second instance of this disease in a dog was reported by Plummer²⁴ and by Radmore²⁵ in 1941. The animal was a male English Setter, 2 years old, domiciled at Hull South, Province of Quebec, Canada. The animal had never been away from that vicinity but had been mated with a female from California. Symptoms on first examination, as recorded by Plummer, included anorexia, emaciation, loss of activity, and reluctance to obey his master's call. Respiration and pulse were rapid; temperature was 103.2° F. (about 1° above the normal limit).

There was an occasional soft cough. Function of the bowels was normal and the feces were negative for parasitic ova. Urine was negative for albumin, sugar, casts, and cells. The red blood cell count, hemoglobin, and clotting time were within normal limits but there was a marked leukocytosis of 21,000 per cmm. The tuberculin test was negative. Within a week after the first examination the dog was much worse, showing symptoms of disturbance of the central nervous system. His eyelids drooped. He circled to the right, bumped into objects, refused food, and had spells of vomiting and polydipsia. He would roam aimlessly, usually at a walking pace, and, while so engaged, was struck and killed by an automobile 1 month subsequent to the first examination.

Significant lesions were found in the lungs and brain. The former were described (by Plummer²⁴) as mottled, firm, and somewhat friable. Resemblance to tuberculosis was noted both grossly and microscopically, although acid-fast organisms were absent. Partaking in the inflammatory reaction were endothelial cells, lymphocytes, plasma cells, a few polymorphonuclear leukocytes, and numerous giant cells. Coccidioidal spherules were present in great numbers, sometimes free but usually within giant or endothelial cells.

The dog's brain showed an olive-shaped nodule, 2 by 1.4 cm., light in color and firm in texture, which was thought to be a glioma. (For this reason no bacteriologic studies were made.) Microscopically, the nodule consisted of epithelioid cells, with fibrous tissue in the deeper parts as well as round cells and a few polymorphonuclear cells. Coccidioidal spherules were present, although less numerous than in the lungs. They were diagnosed by several consultants as *Coccidioides* on the basis of structure and the absence of budding, for which a careful search was made. The third case of coccidioidal granuloma in a dog is reported in this paper.

REPORT OF CASE

The subject was a female Fox Terrier, 7 years old in the spring of 1946. History supplied by the owner was to the effect that the dog was a native of central Iowa but had been living with her owner at an airfield at Eagle Pass, Texas, from the autumn of 1943 to the spring of 1945. During her stay there she was twice under the care of a veterinarian for an ailment which was diagnosed as a throat infection, and which caused dysphagia and loss of appetite. The animal's general health began to decline about 3 months after her return to Iowa, which was 8 months before her death in March, 1946.

The signs and symptoms upon hospitalization were doubtless attributable principally to nephritis but will be summarized briefly as follows: Stiffness and trembling with evidence of abdominal pain; inappetence, vomiting, polydipsia, frequent urination; pulse and respiration, accelerated; temperature, normal; red blood cells, 4,000,000 per cmm.; white blood cells, 24,000, of which 86 per cent were segmented polymorphonuclear leukocytes; hemoglobin, 9.64 gm. per 100 cc. (normal,

Urinary sediment (H. E. stains) shows, in total albumen, a plus, with large numbers of pus cells and many cocci, and a few yeasts, but no acetone, bile salts, and no blood. Culture of the urine on 1% streptococcus agar and *Streptococcus* media shows numerous colonies of streptococci in culture. The animal was treated with 100 mg. of penicillin daily, but the infection was not cleared up. The animal was killed on 10/10/41. The following bacteria were isolated, but the culture of the urine was not cleared up. A purulent vaginal discharge, a few pus cells, and a few yeasts were found in the urine, but no developed. Consequently, the infection was not cleared up.

At post-mortem examination a severe chronic purulent nephritis was found associated with lesions of the liver, lungs and spleen presented lesions unobscured from the clinical picture. Widely scattered through the liver were several in places small, sharply demarcated nodules of white or slightly gray tissue. They were irregular in outline, with a maximum diameter of about 1.5 cm. They were firm and dense to the touch, giving the appearance of connective tissue. No pus or other material could be squeezed out of the cut surface. The lesions were always in close relation to the larger bile ducts and blood vessels; at autopsy they were suspected of being manifestations of a neoplastic change in the walls of the bile ducts. There were three similar structures in the lungs, of no significant size or location, and the spleen contained one nodule, almost perfectly spherical and about 6 mm. in diameter.

Smears from a tube in the liver were negative for acid-fast bacteria. Beyond this, no bacteriologic study was attempted.

Microscopic sections revealed the granulomatous nature of the lesion. The predominant cell was reticulo-endothelial in type, large, usually rather well rounded, and with a very generous amount of acidophilic cytoplasm. The nuclei were pale and vesicular, often indented, and more or less eccentrically placed. Macrophagic activity was evidenced by the rather frequent occurrence of lymphocytes and polymorphonuclear leukocytes within these cells. Polymorphonuclear neutrophils and lymphocytes also were important elements in the reactive tissue. Very commonly all three types of cells were intimately and rather uniformly intermingled, but in other areas there was a tendency for the polymorphonuclear cells to be gathered together to form tiny purulent foci. There were no giant cells and no necrosis or calcification. Stroma, or connective tissue elements, were minimal and inconspicuous, although small, well filled blood vessels were rather numerous. Sharp boundaries separated the granulation tissue from the normal structures which it was invading, but encapsulation, as a rule, did not occur. In the lung there were some areas where the new tissue was almost entirely fibrous, but even here it did not encircle the reticulo-endothelial areas.

Occasionally there was seen a spherule in one stage or another of the

developmental cycle of *Coccidioides immitis*. These were not numerous; most sections contained several spherules, but, of 129 sections examined carefully, 24 showed none. The forms most frequently encountered were simple spheres with a thick, refractile, doubly contoured wall and indistinct, amorphous contents. These were approximately 26 to 28 μ in diameter. More rarely a spherule was found in which endospores (ascospores) were formed or in the process of formation. These had thinner outer walls and varied from 35 to 40 μ in diameter. The first endospores to develop always formed a circle just inside the outer wall; in an equatorial plane about a dozen endospores made up this circle. At a more advanced stage additional endospores filled the center, so that some maternal spherules (asci) were seen completely filled with endospores. At what was doubtless a later stage, the outer cell wall had disappeared and the endospores were beginning to scatter. This scattering was observed to take place at two or three places around the circumference, suggesting that the outer wall had simply dissolved, although the usual conception seems to be that it bursts. Representative of a somewhat later period were irregular clumps of small, thin-walled spherules, each measuring about 6 μ in diameter, recently launched on their new existence as separate individuals. How many were successful in colonizing distant areas of the host's tissues is uncertain, but the fact that the larger forms are usually single leads to the surmise that the process of dissemination takes them some distance from their "birthplace," and, perhaps, that they suffer considerable mortality in this phase of their existence.

COMMENT

It seems possible only to speculate on the route by which the infecting organisms gained entrance to the host's tissues. The respiratory tract is commonly incriminated in this respect and it may be that the pulmonary infection, in spite of its limited extent, was the primary site, with hematogenous spread to the liver and spleen. Opposed to this view is the much more extensive involvement of the liver. The granulomatous areas in that organ were not only more numerous but larger. To interpret the tendency of the hepatic lesions to be centered around large vessels and ducts as indicative of an ascending biliary infection is hardly compatible with the known propensities of this organism. But it points as much toward entry through the portal circulation as through the hepatic and general arterial system. On the other hand, at least some degree of access to the general arterial circulation is necessary to explain the single metastasis found in the spleen.

In view of the usually restricted geographic distribution of coc-

cidoidal granuloma, it is easy to believe that this dog acquired the infection during its stay in Texas. If such is the case the disease required a period of between 1 and 2½ years to attain the condition seen at necropsy. It should be noted, however, that the infection has been reported from the states which border Iowa on the west, south, and east. Sooner or later the first case could be expected to arise in Iowa; perhaps this is it.

Still more difficult to reconcile with the usual geographic distribution is the case of Plummer²⁴ and Radmore.²⁵ Either their dog picked up the infection in the Province of Quebec or else it acquired the disease by contact with the California dog (supposedly healthy) with which it was mated.

SUMMARY

Nearly 100 cases of coccidioidal granuloma in cattle have been recorded, one in a sheep, two in captive wild animals, and two in dogs. A third case in a dog is described. This animal lived in Iowa, but there was a possibility that infection was acquired in Texas.

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DESCRIPTION OF PLATES

PLATE 50

- FIG. 1. External surface of dog's liver, from the new case reported.
- FIG. 2. Cut surface from the same liver. The white areas are granulomatous.
- FIG. 3. The *Coccidioides* spherule in the simple, nonsporulating stage. $\times 620$.
- FIG. 4. Endospores are developing just inside the outer wall. $\times 620$.
- FIG. 5. Endospores have formed only subperipherally, but dissolution and dissemination appear to be taking place. $\times 620$.
- FIG. 6. Endosporulation is nearly complete. Polymorphonuclear leukocytes and reticulo-endothelial granulation tissue are seen. $\times 620$.

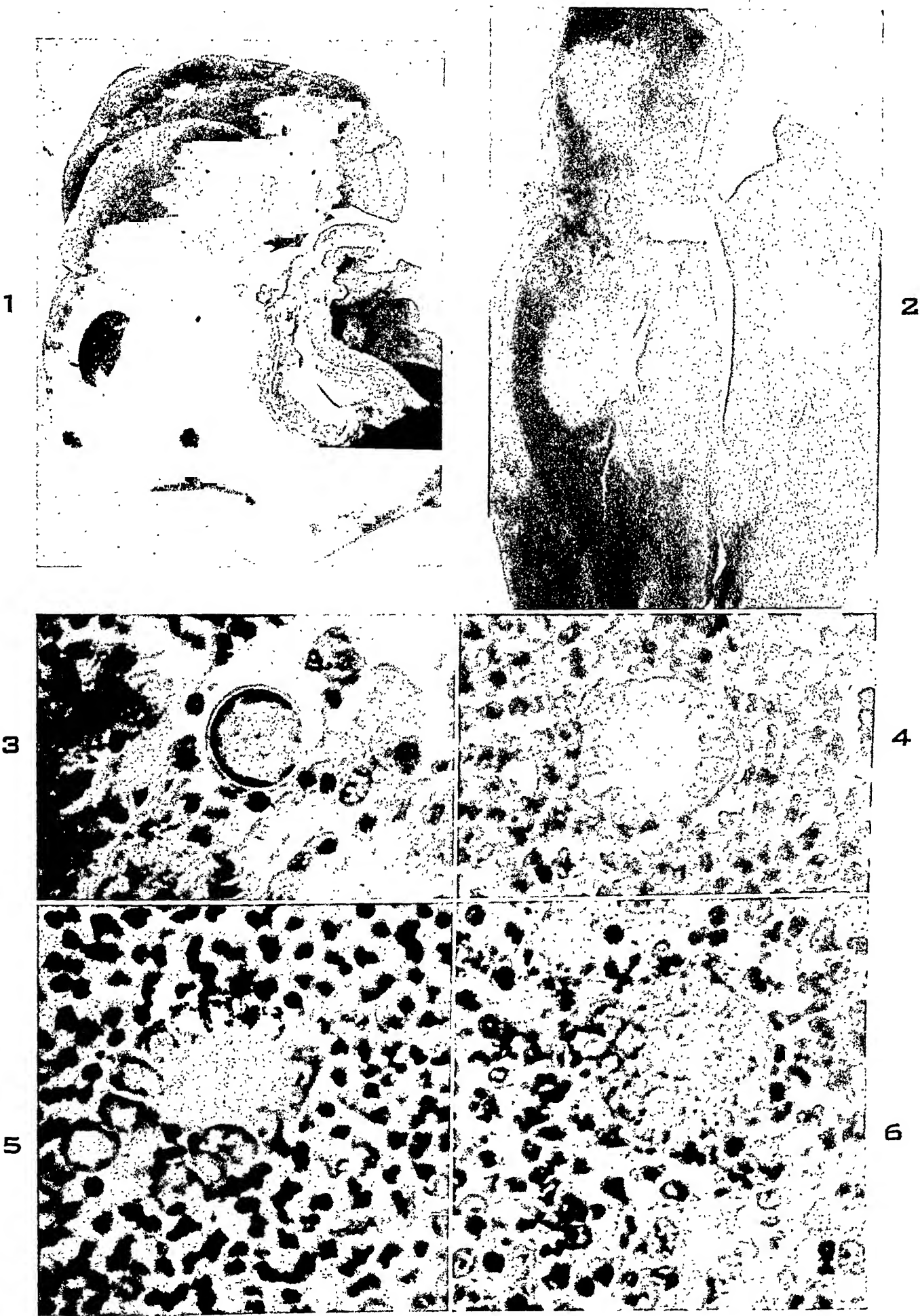


PLATE 51

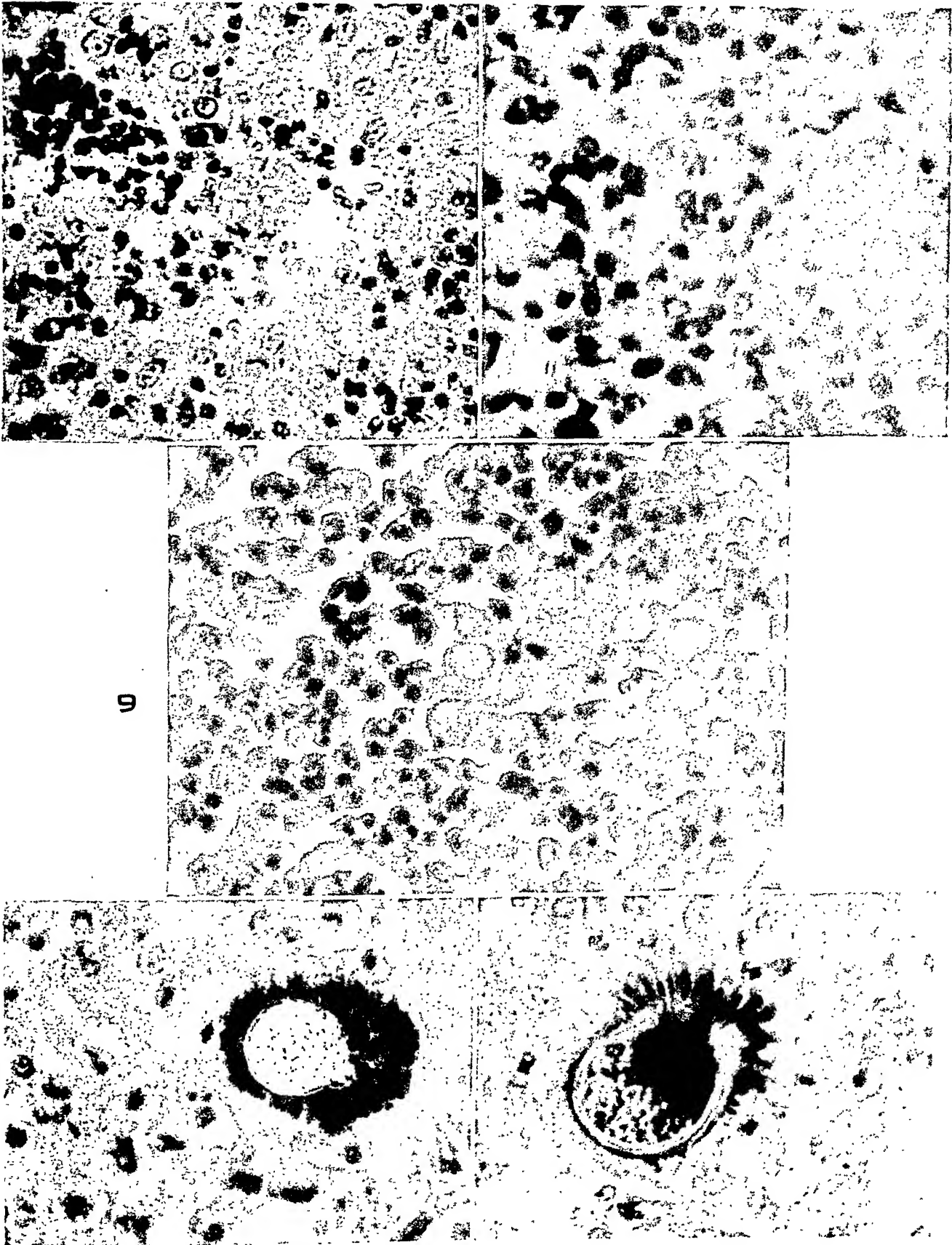
FIG. 7. Endosporulation is complete. $\times 450$.

FIG. 8. The outer wall has dissolved, releasing the endospores. $\times 620$.

FIG. 9. Endospores are beginning to scatter. $\times 620$.

FIG. 10. Rosette-like "clubs" surrounding *Coccidioides immitis*. These clubs stain bright red with hematoxylin and eosin. From a bovine case. (Tissue through the courtesy of Dr. C. L. Davis, Pathological Laboratory, U.S. Bureau of Animal Industry, Denver, Colorado.) $\times 620$.

FIG. 11. Another rosette-like formation of "clubs," only partially encircling the *Coccidioides* organism. (Bovine case from Dr. C. L. Davis.) $\times 620$.



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THE PATHOLOGY OF SECONDARY SHOCK*

VIRGIL H. MOON, M.D.

(From the Army Institute of Pathology, Washington, D.C., and the Department of Pathology, Jefferson Medical College, Philadelphia, Pa.)

The term *secondary shock* is used here to imply a distinction from the uncomplicated effects of hemorrhage—*hemorrhagic shock*—and from *primary shock*. Hemorrhage is an obvious cause for low blood pressure and circulatory deficiency after injuries. Although the clinical signs are similar, the accompanying physiologic changes and the post-mortem findings after death from uncomplicated hemorrhage differ in several ways from those of secondary shock.¹

Primary or "neurogenic shock" (Blalock²) is a neurovascular reaction like syncope or fainting. It may be excited by pain, emotional reactions, or perhaps by nerve impulses arising in damaged tissues (Phemister³). Primary shock develops promptly and usually is transient unless accompanied by extensive injury or hemorrhage; then it may merge into secondary shock with hemorrhage as a contributory factor. Combinations of neurogenic, hemorrhagic, and secondary shock in the same patient have caused confusion.⁴ When the term shock is used without qualification in the succeeding pages, it will be understood to mean secondary shock, sometimes called collapse or peripheral circulatory failure.

DYNAMICS OF SHOCK: RESUMÉ

Shock from wounds was a major problem during World War I. An investigation by eminent physiologists, pharmacologists, internists, and surgeons compared observations on wounded men with data from experimental studies. The results were summarized⁵ as follows:

"The theory of secondary shock which has the strongest support, both in clinical observations and in laboratory experiments, is that of a toxic factor, arising from damaged and dying tissue and operating to cause an increased permeability of the capillary walls and a consequent reduction of blood volume by escape of plasma into the lymph spaces. Thus the concentration of the corpuscles is also readily explained. It is recognized that after a sufficient time infection may occur and be

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of such character in itself as to induce a persistent low blood pressure. According to this theory there might be no essential difference between the effects of toxins given off by damaged tissue and of toxins resulting from activity of bacteria."

Later investigations by many workers indicated the importance of hemorrhage and loss of blood and fluid in traumatized regions. No evidence of toxic substances in the blood was found by the methods then in use. Many believed that all of the phenomena of shock could be explained by local loss of blood and fluid.

The first published report on the pathology of shock ⁶ indicated that hyperemia and edema of viscera, petechial hemorrhages, serous effusions, and acute degeneration of parenchymatous tissues are pathologic changes characteristic of this condition. These changes in visceral areas, remote from the region of trauma, indicated the effects of factors other than loss of blood and fluid. Our subsequent investigations indicated that the syndrome of shock occurs also after burns, poisoning, severe infections, and intestinal obstruction. The same pattern of visceral changes was seen in men and animals after death from these conditions.

The dynamics of shock was interpreted in accordance with the principles of capillary physiology (Krogh,⁷ Landis,⁸ Lewis⁹). Various agents and conditions injurious to endothelium produce atony and dilatation of capillaries and venules. This increases the volume capacity of the vascular bed. Endothelium, when affected by such agents, including anoxia, becomes abnormally pervious to colloids. This allows plasma to escape into the tissue spaces, causing edema and a tendency to hemoconcentration. The loss of plasma lowers the *total* blood volume, and the stagnation of blood in dilated vessels lowers the *effective* blood volume. This leads to a disparity between the volume of blood and volume capacity of the vascular bed, and diminishes the return flow of venous blood to the heart. These effects produce a circulatory deficiency characterized by decreased blood volume and volume-flow of blood, reducing the amount of oxygen delivered to the tissues. Tissue anoxia *per se* causes capillary permeability⁸ and thus introduces a self-perpetuating factor which causes the circulatory deficiency to increase progressively. This vicious circle, unless interrupted, leads to an irreversible stage and to death. It appears that capillary permeability and anoxia have reciprocal effects; either of them presently brings the other into action. Abnormal permeability of endothelium deranges fluid balance. A tendency to visceral edema, hemoconcentration, and to stasis results.

Subsequent investigations indicated the presence of some substance, absorbed from areas of tissue injury, which produces vasodepressor

effects. Best and Solandt,¹⁰ by an exchange transfusion of blood between traumatized and normal dogs, produced circulatory deficiency in the untraumatized animals. Kendrick, Essex, and Helmholtz¹¹ reported similar results from transfusion by a different technic. Freeman, Cullen, and Schecter¹² produced shock by trauma to limbs which had been taped to prevent excessive loss of blood and fluid locally. They found evidence of a toxic factor absorbed from the traumatized region. Similar results were obtained by others when shock was induced by tourniquet and by freezing.

Blalock¹³ produced massive pressure on the muscles of the limbs, simulating the "crush syndrome." Lymph from the thoracic duct, injected into other dogs, caused hemoconcentration and a decline in blood pressure. He believed toxic substances, arising in the injured extremity, had entered the lymph vessels. Experimental crush injury¹⁴ caused hemoconcentration and a fall in blood pressure, accompanied, in some instances, by hematuria and retention of nitrogenous wastes in the blood. Katzenstein, Mylon, and Winternitz¹⁵ found that lymph from the thoracic duct during tourniquet shock caused a protracted fall in blood pressure, often ending fatally, when injected intravenously into other dogs.

Aub and associates¹⁶ showed that bacterial contamination of injured muscles produced toxic substances in the lymph collected from those muscles. Prinzmetal, Freed, and Kruger¹⁷ induced shock by excising and grinding muscle tissue aseptically, then implanting this pulp into the bed from which the muscle was excised. This caused death usually within 24 hours. They believed that shock-producing substances, resulting largely from bacterial contamination, were absorbed from the crushed muscle. It is recalled that all open traumatic wounds are grossly contaminated, and that bacterial growth proceeds rapidly in crushed devitalized tissues.

Summarizing the problems of shock, Blalock¹⁸ found general agreement that traumatic shock is due to regional loss of blood, toxemia, or nerve impulses, separately or in combination. He stated that the search for and identification of the toxic factor or factors is the problem of first importance. Among other problems in the pathogenesis of shock, he listed that of determining the rôle of infection superimposed upon injury.

Shorr, Zweifach, and Furchgott¹⁹ made tests for vasodepressor and vaso-excitor substances in blood and fluids from injured and from anoxic tissues. Their results led to the conclusion that the development of shock is due to vascular atony resulting from a preponderance of vaso-

depressor effects, and that anoxia is a factor causing the circulatory deficiency to progress in a "morbid cycle" to an irreversible stage. Heilbrunn and co-workers²⁰ demonstrated that water extracts of muscle and of tissues from heat-killed animals contained some dialyzable toxic substances which caused death when injected into other animals. Before death these animals showed symptoms like those of heat-treated (scalded or burned) animals.

Recent contributions, as noted above, support the explanation of *traumatic toxemia*, but with important modifications and additions:

(1) It has been demonstrated that, in traumatic or surgical shock, local loss of blood and fluid plays a rôle not recognized formerly. The importance of this factor is in direct proportion to the volume of blood and/or fluid lost. In some instances this is so great as to make it the dominant factor. In other cases it may be minor.

(2) Anoxia is highly important in the vicious circle by which shock tends to progress to an irreversible stage. The anoxia may be *anoxic* as in asphyxia, or *anemic* as when the red corpuscles have been reduced below physiologic limits by hemorrhage or otherwise, or *stagnant* as by circulatory stasis, or *toxic* as from the effects of poisons upon the tissue cells (Blalock²). In any instance, anoxia tends to cause atony and permeability of capillary walls resulting in progressive circulatory deficiency and death. Anoxia is the factor which ultimately "stops the machine and wrecks the machinery."

(3) Comprehension of the mechanism of shock has led to the recognition of the same syndrome in conditions other than trauma, extensive surgery, and burns. Atchley²¹ cited instances in patients with severe infection, diabetic acidosis, Addison's disease, bile peritonitis, vaccine reactions, heat prostration, and snake bites. These conditions led to stasis of blood in the capillaries, generalized anoxemia, capillary dilatation, and leakage of plasma into the tissues, operating in a vicious circle. Harkins²² stated that shock may occur in association with a wide variety of conditions: hemorrhage; mechanical or operative trauma; burning; freezing; heat stroke; radiation burns; sunburn; asphyxia; vascular occlusion; intestinal strangulation; application of tourniquet; bile peritonitis; perforated gastric ulcer; pancreatitis; various poisons such as HgCl_2 , arsenic, gold chloride, snake venoms; special capillary poisons such as products of tissue autolysis, histamine, peptone; medical conditions such as an anaphylaxis, diabetic coma, eclampsia; severe infections such as cholera, streptococcal and influenzal pneumonia, gas gangrene, diphtheria, peritonitis; and various anesthetic agents.

A condensed summary on the mechanism and associated features was issued by the Subcommittee on Shock ²³ of the National Research Council. It set forth that the outstanding physiologic feature is peripheral circulatory failure caused by a discrepancy between the capacity of the vascular system and the volume of fluid which it contains. Shock was defined as the *clinical condition characterized by progressive reduction in circulating blood volume due to increased capillary permeability*. Any agency which affects the permeability of the vessels allowing the escape of plasma proteins, may lead to shock. This is accompanied by a progressive reduction of circulating blood volume due to the escape of plasma. Anoxia is a factor of prime importance causing capillary permeability and impaired circulation. This summary closed with a brief statement on associated tissue changes:

"The pathologic picture which is found when the tissues are examined after death from shock is that to be expected from peripheral circulatory failure. There is widespread congestion and engorgement of the capillaries and venules throughout the body This congestion is found throughout the viscera and in the lungs. There is edema in the tissue spaces and effusion in the serous cavities The impairment of circulation affects other organs as well. Necrosis of liver cells, liquefaction of the suprarenal medulla, and congestion of the pancreas are observed. The kidneys show evidence of parenchymatous degeneration. Patches of capillary hemorrhage occur in the medulla, and numerous red cells are found within the tubules in such regions. The pathologic picture thus confirms the clinical and experimental evidence on the significance of increased capillary permeability as the essential feature of shock."

The preceding data indicate that divergent views have been adjusted concerning the major factors in the dynamics of shock. Investigations on other features can now go forward relatively undisturbed by controversial discussions.

MATERIAL AND METHODS

The purpose of this survey is to secure information on the occurrence of shock from causes other than trauma, to collect additional data on associated pathologic changes, and to record evidence of physiologic disturbances, especially the renal effects.

The Director of the Army Institute of Pathology provided opportunity to study the material collected there. The clinical and post-mortem records, histologic preparations, and the entire facilities of the Institute were made available for a survey on the pathology of shock as seen in the personnel of the U. S. Army. Thousands of records of death from all causes presented an abundance of material for study. To tabulate the data from all of these was an undertaking of staggering proportions, for which time was not available. Accordingly, the statistical approach was abandoned in favor of detailed studies of representative cases appropriately grouped. In every instance the

clinical and post-mortem data were taken directly from the records, but the microscopic studies were made by me.

TRAUMA

Under the heading of trauma are grouped those instances in which fatal circulatory deficiency resulted from traumatic or surgical injuries. The causative mechanism in such cases includes trauma, hemorrhage, infection (all battle wounds are grossly contaminated), and sometimes anesthesia, surgery, reaction to transfusions, renal complications and other contributory conditions. It is manifestly impossible to evaluate the relative weight of these several factors in any given case. The causation of shock in this group is more complex than in the succeeding groups.

Thirty cases of shock resulting from trauma and associated conditions were studied. They presented different degrees of severity as indicated by the interval between injury and death, ranging from a few hours to 6 days. These features are indicated briefly in the list which follows:

A.I.P. Accession Number

Origin

84837	Abdomen hit by the recoil of a 90 mm. gun. Death 8 hours later.
122323	Death during surgical operation for G.S.W.* received weeks before.
122486	Death during surgical operation for multiple G.S.W.
128121	S.F.W.* of thighs, compound fracture, transfusion reaction, anuria.
126488	G.S.W. of legs and feet, compound fracture. Death 24 hours later.
122293	G.S.W. of thigh, fractured femur. Death 24 hours later.
86750	G.S.W. of body, fractures. Death 24 hours later.
114945	G.S.W., multiple fractures. Death 30 hours later.
88192	Automobile accident, multiple fractures, gas gangrene. Death 34 hours later.
86134	Fall from fourth story window, compound fractures. Death 36 hours later.
97750	Automobile accident, fractures, internal injuries. Death 48 hours later.
118315	S.F.W. of thighs, pulmonary edema. Death 48 hours later.
118316	G.S.W. of leg and thigh, fractures. Death 48 hours later.
127136	S.F.W. of legs and feet, compound fracture, anuria. Death 48 hours later.
84641	Automobile accident, internal injuries, anuria, high nonprotein nitrogen. Death 53 hours later.
85677	Automobile accident, fractures of femurs, mandibles, ribs. Death 3 days later.
126438	G.S.W. of arm, fractures, gas gangrene, amputation. Death 3 days later.
127580	S.F.W. of buttocks, pulmonary edema, anuria. Death 3 days later.
102060	Resection of rectal carcinoma, oliguria, uremia. Death 4 days later.
119159	Automobile accident, compound fracture of pelvis. Death 4 days later.

* G.S.W. and S.F.W. indicate gunshot wounds and shell fragment wounds, respectively.

122144	S.F.W., comminuted fractures of legs and arms, gas gangrene, oliguria. Death 4 days later.
113425	Ileitis, surgical resection, anuria, high nonprotein nitrogen. Death 5 days later.
118404	S.F.W. of thighs and leg, compound fractures, anuria, high nonprotein nitrogen. Death 5 days later.
121439	G.S.W., transfusion reaction, anuria. Death 5 days later.
118313	G.S.W. of legs, transfusion (reaction?), high nonprotein nitrogen. Death 6 days later.
121411	S.F.W. of legs, comminuted fractures, shock developed 6 days later.
128117	S.F.W., multiple fractures, anuria, uremia. Death 6 days later.
126031	S.F.W., gas gangrene, amputation. Death 6 days after receipt of wound.
127269	G.S.W. of leg, ischemic gangrene. Death 6 days later.
118359	S.F.W. of buttocks and legs, fractures, gas gangrene, amputation. Death 10 days after receipt of wound.

Pathologic Conditions

Pulmonary hyperemia and edema were found regularly in all cases. In most instances these were marked; they were slight or moderate in only 6. Serous effusions were present in 21. Petechiae were noted at necropsy or found microscopically in 15. The development of pneumonia in shock with delayed death has been reported.²⁴ In 20 cases death occurred 48 hours or longer after the injury; terminal pneumonia was present in 12 of these, either incipient or well advanced (Figs. 1 and 10).

Some degree of atelectasis was present in 13 cases. In some this was noted grossly at necropsy; in others it was seen in varying degrees microscopically, as small scattered areas. This feature has not been reported hitherto as associated with shock. Its significance and mechanism of origin are not apparent.

Parenchymatous degeneration ranging to necrosis was a regular feature, especially in the kidneys, liver, and heart. In 20 cases, marked parenchymatous degeneration was present in the kidneys; in 12 of these, necrosis of tubular epithelium also was present; in 6 cases the degeneration was moderate or slight. In the remaining 2 instances, post-mortem changes made the recognition of degeneration uncertain. Casts were present in the tubular lumina in 19 instances; these were hyaline, granular, cellular, or mixed; in a few instances the casts contained brown pigment. In all instances the renal parenchyma was moderately or markedly hyperemic; minute hemorrhages were seen frequently, and red cells were often found in the tubular lumina (Figs. 4 and 5).

In 26 cases, parenchymatous degeneration of hepatic cells was marked; in 22 there was also necrosis. This varied from occasional scattered cells, focal necrosis, to extensive areas usually in the central

part of the lobules (Fig. 3). Degenerative changes of heart muscle fibers, ranging from slight to marked, were present in 21 instances. In a few cases foci of necrosis were present in lymphoid tissue and in the adrenals (Fig. 2). Splenic engorgement was noted in 13 instances.

In these cases, shock resulted not from a single causative agent but from a combination of causes: the delayed effects of tissue injury, hemorrhage, infection, and, in some instances, anesthesia, surgery, and unfavorable reaction to transfusion. The relative importance of these factors varied from case to case.

Hemorrhage and local loss of blood had occurred in many cases. That hemorrhage was not the sole factor was suggested by the facts: (1) that restoration of blood volume by fluids, plasma, and transfusion did not restore circulatory efficiency; (2) that in some instances a high red blood cell count indicated that some mechanism had disturbed fluid balance and had prevented the dilution of blood which normally follows hemorrhage; (3) that marked visceral congestion, edema, effusions, and petechiae indicated the effects of some other agency. These findings are not characteristic of death from uncomplicated hemorrhage.

In some instances infection was not apparent from the clinical or post-mortem data, while in battle wounds, some complicated by gas gangrene, infection was a prominent contributory factor. The visceral findings in these were like those seen throughout the group. There is evidence that products of bacterial growth may cause capillary permeability. Examinations of the blood, when recorded, showed varying degrees of hemodilution; in only 3 instances was there hemoconcentration. However, most cases had received fluid intravenously which would, of course, counteract hemoconcentration. In some, the large amounts of fluid given were more, perhaps, than a failing circulation could retain. Some degree of capillary permeability is a central feature in shock; such capillaries will allow saline fluids to escape rapidly, thus increasing the edema.

The circulatory disturbances which lead to shock may develop in varying degrees and with varying rapidity. Maximal degrees may cause death from failure of the circulation within 24 hours regardless of therapeutic measures. With lesser degrees the patient may respond favorably and recover; in others, a condition of sublethal shock persists for several days. In such cases, either renal insufficiency or terminal pneumonia may be expected to complicate the condition.

Oliguria or anuria is present early and continues as shock progresses (Atchley,²⁵ Freeman,²³ Blalock²). This often leads to uremia if the subject survives for a few days.²⁶ Renal deficiency and retention of nitrogenous wastes were present in 8 cases of this group and 3 of these followed transfusion reactions. A cause for renal insufficiency is suggested in the marked degeneration and necrosis of the tubular epithelium.

Transfusion reactions vary widely in degree. The severest reactions cause death from progressive circulatory collapse, sometimes resembling fatal anaphylactic shock (Best and Taylor,²⁷ Bordley,²⁸ Wiener²⁹). Renal deficiency develops following severe nonfatal reactions and death may occur from uremia, as in cases 121439, 127136, and 128121. In these the visceral findings were the same as in delayed death after shock from other causes. Degenerative changes and necrosis of renal tubular epithelium were marked, as in other instances of uremia associated with shock.

Traumatic shock results from a combination of causes, the relative importance of which varies in different cases. The causative factors in subsequent groups do not include so many variables, hence an analysis of the mechanism will be less involved. Early death from traumatic shock results from circulatory failure. Death after 48 hours often is the result of a combination of circulatory deficiency with renal deficiency and terminal pneumonia.

BURNS

Patients with extensive burns of the skin often present the complete picture of secondary shock. Harkins'³⁰ survey indicated that shock is the cause of death in 60 to 75 per cent of fatalities from burns. Examination of data from numerous cases indicated that they confirm what is already recorded in medical literature concerning the clinical course and the post-mortem findings after death from burns. Ten representative cases were studied for comparison with the findings in shock from other causes. Also 3 instances of burns made by phosphorus upon the skin or blown into the flesh were studied. In these, absorption introduced a possible factor of phosphorus poisoning.

A.I.P. Acces-

sion Number Conditions

87628	Extensive 2nd and 3rd degree burns. Death 12 hours later.
122578	Extensive 2nd and 3rd degree burns, about 40 per cent of skin. Death 12 hours later.
125364	Extensive 2nd and 3rd degree burns. Death 15 hours later.

125903	Extensive 2nd and 3rd degree burns. Death 24 hours later.
107676	Extensive 3rd degree burns, high nonprotein nitrogen. Death 28 hours later.
86891	Fractures, 2nd and 3rd degree burns. Death 26 hours later.
127272	Extensive 2nd and 3rd degree burns. Death 48 hours later.
122679	Burns of entire body, anuria. Death 3 days later.
90393	Extensive severe burns, anuria. Death 4 days later.
118506	Extensive 2nd and 3rd degree burns, high nonprotein nitrogen. Death 8 days later.
114072	Phosphorus burns, 40 per cent of skin. Death 12 hours later.
93453	Phosphorus burns, 2nd and 3rd degree. Death 15 hours later.
106660	Phosphorus burns, 2nd and 3rd degree, anuria, high nonprotein nitrogen. Death 4 days later.

In every case the findings were like those of traumatic shock, but tended to be more severe. Pulmonary hyperemia and edema were marked (Fig. 6). Pleural effusions were noted in 7, and petechiae in 9 instances. Terminal pneumonia was present in the lungs of 3 men who lived longer than 2 days after burning. The degeneration and necrosis of renal tubular epithelium were marked; degeneration and necrosis of hepatic cells were extensive (Fig. 9). In 10 instances the adrenal cortices were degenerated and abnormally vacuolated. In a few instances, post-mortem changes made the recognition of degeneration uncertain. Myocardial degeneration was seen in 12 of the 13 cases. Casts were found in renal tubules in 7 instances. In one of these the casts contained quantities of brown pigment; no transfusion or sulfonamide therapy had been given in this case (Figs. 8 and 9).

Some writers disregard the systemic effects of burns and attribute depleted blood volume and hemoconcentration to local loss of fluid in and about the burned areas. The importance of such loss is unquestionable; its effects are proportional to the amount of fluid lost. But loss of fluid locally does not account for the intense hyperemia, edema, acute degeneration and necrosis occurring in visceral areas remote from the burn. These indicate widespread and serious *systemic* effects. No treatise on burns will be complete unless it provides an acceptable explanation for the systemic as well as the local effects.

It has been suggested that degeneration and necrosis may result from the absorption of tannic acid, sulfonamides, or other drugs applied to the burns. It should be remembered that visceral hyperemia, edema, and degeneration and necrosis of hepatic and renal cells were set forth as characteristic features long before such local treatments were practiced (Bardeen,³¹ Pack³²). Significant evidence has accumulated indicating the systemic effects of toxic products absorbed from the burned tissue itself, and no evidence incompatible with this interpretation has been shown.

POISONING

It has been observed that acute poisoning often is accompanied by signs of shock. These include weakness, perspiration, vomiting, rapid pulse, low blood pressure, and oliguria. Hemoconcentration usually accompanies this syndrome.³³ Often the visceral changes, including hyperemia, parenchymatous degeneration, edema, and petechial hemorrhages, are like those already described. These effects result from various poisons, hence they are not the specific effect of some particular drug or chemical. It is of interest to compare the changes seen in the viscera after death by poisoning with those seen after shock from trauma and from burns. Data and post-mortem evidence from 20 cases of poisoning were studied.

*A.I.P. Acces-**sion Number Conditions*

89731	Shock reaction, delayed 3 days after neoarsphenamine. Death 24 hours later.
111925	Immediate reaction to mapharsen, oliguria, high nonprotein nitrogen, hemoconcentration, uremia. Death 7 days later.
120574	Arsenic poisoning, oliguria, high nonprotein nitrogen, hemoconcentration. Death 36 hours later.
128449	Suicide by arsenic, shock. Death same day.
129165	Suicide by arsenic, shock, hemoconcentration. Death 28 hours later.
60482	Mercuric poisoning, anuria, hemoconcentration. Death 2 days later.
66984	Suicidal mercuric poisoning, anuria, high nonprotein nitrogen. Death 8 days later.
98365	Mercuric poisoning, shock, hemoconcentration. Death 24 hours later.
72500	Suicide, rat poison (phosphorus). Death 7 days later.
85342	Suicide, rat poison (phosphorus). Death 9 hours later.
98461	Suicide, rat poison (phosphorus), shock. Death 5 days later.
103527	Overdose of barbiturate. Death 24 hours later.
106299	Found dead, barbiturate poisoning.
106544	Seconal poisoning, shock, found unconscious.
108088	Found dead, barbiturate poisoning.
116989	Suicide by barbiturate. Death 2 days later.
113935	Alcoholism and barbiturate poisoning. Death 48 hours later.
128452	Suicide by barbiturate poisoning, shown by chemical analysis.
128656	Barbiturate poisoning, shown by chemical analysis.
128611	Protracted drinking bout, acute alcoholism.

Pulmonary hyperemia and edema were present in marked degree in 13; in 6 they were slight or moderate; and in one, suicide by arsenic, they were absent. Atelectasis was seen in 4 instances. Terminal pneumonia was found in 4 of the 7 men who lived 48 hours or longer. Petechial hemorrhages were recorded in 12, serous effusions in only 4. Degeneration and necrosis of renal tubular epithelium were found regularly, regardless of the type of poisoning. Necrosis was more marked after poisoning with arsenicals, mercury, and phosphorus than

after barbiturate poisoning; also, fewer casts were found in the tubules in the latter. Likewise degeneration and necrosis of hepatic cells were regularly present, but were less marked after barbiturate poisoning. Myocardial degeneration was seen in 13 cases; in 7 cases no histologic section of heart muscle was saved. Splenic engorgement was present in 13 cases.

Traumatic shock results from the combined effects of several causative and contributory conditions, hence the difficulty of accurate analysis, but shock from the effect of poisons is less complicated since the contributory factors are few. There is no possibility of nerve impulses from wounded tissues, of hemorrhage or leakage of plasma locally, of infection, anesthesia, or reaction to transfusion of blood or plasma. These indeterminate variables have been eliminated from the equation, making analysis relatively simple.

Students of capillary reactions ^{7,9,34} have shown that various drugs and chemicals, sometimes called "capillary poisons," may cause atony, dilatation, and increased permeability of capillary endothelium. Any kind of injury to capillaries increases the permeability of their walls.⁹ These considerations indicate that various poisons may produce shock by their direct effects upon capillary endothelium. This interpretation is supported by the visceral findings; the capillovenous hyperemia, edema, serous effusions, and petechiae indicate atony, dilatation, and abnormal permeability of the walls of the minute vessels. Such changes did not vary in kind or degree with the various types of poison. The renal effects of poisons, especially mercurials and arsenicals, are well known. These are considered in a later section.

INFECTIONS

Internists have observed that severe fulminating infection may cause progressive circulatory failure like that of shock from other causes. This effect does not depend upon the bacterial species, but upon the virulence in the individual case. Ten cases of severe infection, as listed below, were studied.

A.I.P. Accession Number

Conditions

118865	Fulminating meningitis. Death 12 hours later.
93449	Fulminating meningitis. Death 12 hours later.
106479	Fulminating meningitis. Death 24 hours later.
128538	Fulminating meningitis. Death 24 hours later.
108004	Mixed streptococcal and staphylococcal meningitis.
101617	Septicemia (organism not stated). Death 6 hours later.
108955	Streptococcal septicemia. Death 7.5 hours later.

- 102147 Influenza. Death 48 hours later.
111409 Severe infection (tularemia?), anuria, high nonprotein nitrogen, "uremic frost." Death 12 days later.
125907 *Falciparum* malaria, anuria, high nonprotein nitrogen. Death 9 days later.

The pathologic findings in each of these cases presented the same degeneration ranging to necrosis, as seen in shock from trauma, burns, and from poisons. Pulmonary hyperemia and edema were intense in 9 cases, moderate in one (93449). Petechiae were present in 9 and absent in one (111409). Hepatic, renal, and cardiac degeneration and necrosis were marked in all cases. Two of the 4 cases of fulminant meningitis—Waterhouse-Friderichsen syndrome—had extensive adrenal hemorrhages. In one case these were absent and in one no observations on the adrenals were recorded and sections of them were lacking.

Two patients, cases 125907 and 111409, died of uremia on the 9th and 12th days, respectively. In one, terminal pneumonia also was present. Each showed extensive degeneration and necrosis of renal tubular epithelium, casts, and debris in the tubular lumina. Similar renal findings were present in the patient with influenza dying in 48 hours.

The occurrence of circulatory collapse is not unusual in diphtheria, septicemia, meningitis, gas gangrene, cholera, plague, yellow fever, malaria, and in other acute infections. Its occurrence seems to depend on the severity or virulence of the infection. Dale³⁵ stated that the action of bacterial ferments upon proteins may give rise to cleavage products which will cause secondary shock. Atchley²¹ believed that this results from paralysis of the capillaries by bacterial toxins. He cited an instance of pneumococcal vaccine given intravenously, resulting in shock. Brodie³⁶ found that small amounts of diphtheria toxin caused an immediate fall in blood pressure when given intravenously to animals. MacCallum³⁷ made similar observations. Harding³⁸ surveyed 800 cases of diphtheria and noted that circulatory failure, with hemoconcentration like that of wound shock, occurred in the toxemic stage. Influenza of fulminating severity produces progressive circulatory deficiency ending in death. Other instances might be cited.

Shock in severe infections, like that resulting from poisons, is relatively simple in its origin; trauma, hemorrhage, anesthesia, local loss of fluid and other complicating factors are not involved. Many believe that bacterial toxins, or products of bacterial metabolism in the tissues, affect endothelium like histamine or other capillary poisons. This

interpretation is supported by necropsy findings in such cases. These are not different in kind from those seen in shock from other causes.

It is recognized by internists that severe infections often cause deficient renal function. This occurrence is not limited to yellow fever and falciparum malaria but is seen with other infections. Two of the cases studied showed this feature. The gross and microscopic changes in the kidneys were like those accompanying renal failure in the other groups.

ANOXIA

The importance of *anoxia* in the mechanism of secondary shock has been emphasized by many authors. It is believed to be a major factor in the vicious circle by which shock progresses to an irreversible stage. For this reason, findings after death from simple lack of oxygen, or from asphyxia, are of interest. A group of 15 such cases was studied.

A.I.P. Access-

<i>sion Number</i>	<i>Conditions</i>
83172	Removed O ₂ mask in low pressure chamber, equivalent altitude 36,500 ft.
108338	Removed O ₂ mask in flight at 23,000 ft.
111237	O ₂ line disconnected in flight at 27,000 ft.
114331	O ₂ line disconnected in flight at 30,000 ft.
114333	O ₂ line frozen in flight at 30,000 ft.
114342	O ₂ line disconnected at 31,500 ft.
114334	O ₂ line disconnected at 30,000 ft.
114356	O ₂ tank empty at 26,000 ft.
114357	O ₂ line frozen at 26,000 ft.
100788	Asphyxiated by CO.
128137	Asphyxiated by CO.
128450	Asphyxiated by CO.
129055	Asphyxiated by CO.
127170	Suffocation by fumes in burning building.
128153	Death by drowning.

Ten additional records of death by anoxia in aviation were reviewed. In these the necropsies were done 24 to 48 hours after death, hence observations on parenchymatous changes were unsatisfactory. The circulatory changes in these 10 were exactly like those given below, but figures from these are not included in the findings.

The visceral changes were like those in the preceding groups except that hyperemia was more intense and necrosis and edema were less marked. In each case there was intense hyperemia of the lungs, liver, and kidneys. Petechial hemorrhages were noted in the post-mortem records in each instance. Edema of the lungs was marked in 3, moderate in 8, and absent in 4 cases; serous effusions were not recorded in any instance. Parenchymatous degeneration of the liver and kidneys was marked in 8 cases, slight in one; in 6 instances, post-mortem

changes made the recognition uncertain. Myocardial degeneration was seen in only 7 instances. Some degree of necrosis of both hepatic and renal tubular cells was seen in 8 cases.

The time interval between the beginning of anoxia and the cessation of circulation is not known; however, it probably was short, perhaps 15 to 30 minutes. Perhaps this was insufficient time for the development of edema and effusions. The presence of severe parenchymatous degeneration and of beginning necrosis deserves comment. Since they developed in so short a time, it appears that renal and hepatic epithelium is delicately susceptible to anoxia, even of short duration. It is significant also that petechial hemorrhages were seen in every case.

The cause of death in this group was simple. Neither nerve impulses from traumatized tissues, hemorrhages, local loss of fluid, poisonous substances, bacterial toxins, nor products of tissue autolysis were possible factors. Yet the visceral changes were of the same pattern as when one or more of the factors just mentioned were operative. Anoxia appears to be the one common factor in the mechanism of secondary shock from diverse causes. Perhaps it is the most important cause for petechial hemorrhages and for the parenchymatous changes found after death by shock.

When men succumbed to lack of oxygen in high altitude flight, the additional factor of low atmospheric pressure was introduced. The intense hyperemia, extreme dilatation of capillaries and venules, and the occurrence of numerous petechiae may be due in part to decreased extravascular (atmospheric) pressure. This would tend also to cause edema. Hyperemia, edema, and petechiae can be produced locally by applying a vacuum cup to a normal skin surface.

In the instances of death by drowning, nitrous oxide anesthesia, suffocation, and asphyxia by carbon monoxide, the anoxia was not accompanied by low atmospheric pressure. The same pattern of changes was present in these cases although the congestion and edema were less intense than when anoxia was combined with low atmospheric pressure.

LOW ATMOSPHERIC PRESSURE

Aviation at high altitude often causes collapse resembling surgical shock even though abundant oxygen is supplied. "The most dangerous aspects of severe reactions resulting from exposure to lowered barometric pressure are the latent period of from 1 to 6 hours which frequently precede the manifest appearance of clinical shock; and the consequent resistance to treatment of what may be, at this point, a full-blown vicious circle of peripheral circulatory insufficiency, tissue anoxia, and marked hemocentration."³⁹ Chambers from which the air was ex-

hausted, to simulate atmospheric conditions at various altitudes, were used to test the ability of aviators to withstand the effects of low pressure; oxygen was supplied as in actual high altitude flight. Fatalities occasionally resulted even under most careful supervision of such tests. I had opportunity to study the data and to determine the pathologic findings in 5 such cases for comparison with those of shock from other causes.

A.I.P. Accession Number

Conditions

95412	72 minutes at 38,000 ft. Death 10 hours later.
100822	One hour at 30,000 ft. Death 24 hours later.
100893	Discomfort and pain at 38,000 ft. Death 47 hours later.
103767	Discomfort and pain at 38,000 ft. Death 17 hours later.
127451	20 minutes at 30,000 ft. Death 55 hours later.

Since little is known of the mechanism causing shock from low atmospheric pressure, such cases are of special interest. Accordingly, condensed clinical and pathologic data from 2 representative cases are given.

Case 100822

After 1 hour at 30,000 ft. and while ascending to 38,000 ft., the subject gave up the test because of severe "bends" and "chokes." When taken out of the chamber he was very weak and sweating profusely. He was given oxygen by inhalation, and was placed under observation. The blood pressure was 140/104 mm. Hg; pulse, 90; red blood cells, 5,400,000; leukocytes, 23,000; hematocrit, 60. Roentgenograms of the chest showed pulmonary edema, recorded as moderately severe. Four hours later he was restless and vomited twice. The pulse was 140; the blood pressure, 120/80, later declining to 90/0. Plasma given intravenously caused no increase in the blood pressure; oxygen was continued. The next day he was weaker, restless, and cyanotic. Death occurred about 24 hours after the test.

At post-mortem examination, 750 cc. of serous fluid was found in each pleural cavity. The lungs, weighing 850 and 742 gm., were congested and markedly edematous. The heart weighed 410 gm. and showed no visible abnormalities. The spleen weighed 220 gm. and the liver, 2395 gm., with no abnormal features. Petechial hemorrhages were seen in the gastric mucosa and marked hemorrhages in the lining of the jejunum and ileum. There was congestion of the meninges. The kidneys, weighing 200 and 180 gm., were congested.

Microscopic Examination. The lungs showed intense capillovenous hyperemia and edema. The myocardium was edematous and the fibers showed parenchymatous changes. The spleen was ischemic. The liver showed degeneration, marked fatty changes, and central necrosis. There was intense hyperemia of the gastrointestinal mucosa. The cortical cells of the adrenals were vacuolated. The brain and meninges were hyperemic and edematous. The renal cortex and the medulla were moderately hyperemic. The tubular epithelium showed degenerative

changes which seemed more advanced in the upper than in the lower segments.

Case 100893

At a relative altitude of 38,000 ft., the subject became pale, the respirations were increased, and he complained of muscular pain and weakness. The altitude was reduced gradually and the test was discontinued. The pulse was 60; the blood pressure, 100/60 mm. Hg. The heart sounds were normal, the breath sounds were decreased, and the skin moist. He seemed drowsy and unable to talk coherently.

When placed in the hospital, the pulse was 80; the blood pressure, 110/80. Four hours later he seemed worse, irrational, and was thrashing about. Plasma, saline solution, and oxygen were given, and his general condition improved. The hematocrit at this time was 56.1; the white cells, 20,000. Three hours later his condition became grave, the hematocrit was 64.9, no pulse could be obtained; he was sweating and apparently in profound shock. The extremities were cold but the rectal temperature was 107.6°F.; on recheck 10 minutes later, this was 108.4° F. He was packed in ice and given plasma and saline solution. On the following day the general condition seemed improved but still critical. He was restless and the breathing was labored. The blood pressure was 122/80; hematocrit, 55. He was given plasma and saline solution continuously. On x-ray examination the cardiac shadow was not enlarged, but the pulmonary markings were increased and interpreted as vascular stasis. On the next day the respirations were easier, the blood pressure more nearly normal, and the temperature lower. The hematocrit was 50; white cells, 13,500. The urine showed a specific gravity of 1.017; albumin, 4 plus. He was seized with convulsions, the circulation failed, and death occurred 45 hours after admission to the hospital.

Post-mortem examination revealed marked hyperemia and edema of the lungs. These were described as solid in the posterior portions. The liver and brain were congested. No other gross abnormalities were recorded.

Microscopic Examination. The lungs were intensely hyperemic and edematous. There were areas of atelectasis and capillary hemorrhages (Fig. 16). The myocardium was edematous and degenerated. The splenic pulp was deficient in red blood cells. The liver was hyperemic; the cells showed advanced parenchymatous degeneration ranging to necrosis (Fig. 17). The adrenal cortex was hyperemic; the cortical cells were vacuolated. The brain showed widespread autolysis and a few perivascular hemorrhages. The renal parenchyma was hyperemic. The tubular epithelium was flat and the lumina were wide. Albuminous material was present in the glomerular spaces. The collecting tubules contained *dark, pigmented casts*, granular casts, and erythrocytes. The stroma was edematous (Figs. 18 and 19).

In each of the 5 cases, the gross and microscopic findings were essentially the same as in these 2. Shock from low atmospheric pressure presents some features not seen in shock from other causes: The body temperature was excessively high; there were convulsions and other signs of neurologic disturbances; extensive degeneration was found in

various parts of the central nervous system. In these particulars the syndrome resembles that of heat stroke, as will appear in the succeeding group. Several items were especially noteworthy in these cases: The concentration of the blood was high; in one case the hematocrit reading was 64.9. Other clinical signs of circulatory failure were marked. It is significant that the visceral findings were of the same pattern as in the other groups. The development of renal failure, with progressive retention of nitrogenous wastes, was rapid in case 100893. Pigmented casts and other changes in the kidneys were like those seen in the "crush syndrome"⁴⁰ although no transfusions nor sulfonamide therapy had been employed.

Several items are suggested as possible factors in the mechanism of shock from low atmospheric pressure. Low external pressure would contribute mechanically to the dilatation of minute vessels and to the leakage of fluid through their walls. This might derange fluid balance as seriously as would toxic or anoxic injury to endothelium; hence the hemoconcentration and circulatory deficiency. It has been suggested that low barometric pressure interferes directly with internal respiration even though abundant oxygen is supplied for breathing. Neither oxygen nor other gases under low pressure are absorbed readily by fluids. The hemoglobin of the erythrocytes is essentially a fluid; it may not absorb physiologic amounts of oxygen if the gaseous pressure is greatly decreased. Low external pressure may be directly injurious to the central nervous system. Extensive neurologic damage was found histologically in various parts of the brain. It is known that trauma, embolism, infection, or hemorrhage in some areas of the brain may produce the clinical syndrome of shock accompanied by hemoconcentration and characteristic visceral changes. The exact mechanism of these phenomena has not been shown and should be a fruitful subject for investigation by neurophysiologists.

Once the circulation and fluid balance are deranged, by any one of these mechanisms or by their combined effects, the resulting anoxia in the tissues will perpetuate the vicious cycle of secondary shock with its characteristic visceral and renal effects.

HEAT STROKE

Features of resemblance between heat stroke and secondary shock have been reported.²⁶ Drinker⁴¹ noted sudden collapse and coma accompanied by vomiting and high fever, rapid pulse, and low blood pressure as the usual signs. He stated that the condition resembles surgical shock except for the elevation of temperature. Hill⁴² ob-

served that at necropsy "the organs . . . show capillary congestion, as in wound-shock."

Several authors^{21, 22, 41, 43} include heat stroke among the conditions which may cause shock. Hartman^{44, 45} observed manifestations of shock in cases of therapeutic hyperthermia resulting fatally. In one, the blood pressure sank to 68/20 at the end of the treatment; death came 20 hours later. He stated that the pathologic changes, both in human cases and in experimental animals, were typical of anoxia produced in other ways.

Twelve representative cases of heat stroke were selected in which death occurred after intervals varying from 25 minutes to 6 days; one case following therapeutic hyperthermia is included. Shock had been noted clinically in most cases.

A.I.P. Accession Number

Conditions

84012	Fell unconscious during drill. Death 25 minutes later.
97543	Fell unconscious during hike. Death 3 hours later.
96187	Collapsed, comatose, after 25 mile hike. Death 6 hours later.
112746	Severe sunstroke while on duty. Death 6.5 hours later.
85876	Collapsed during "K.P." duty. Death 12 hours later.
102099	Fell unconscious while marching. Death 24 hours later.
97148	Collapsed during heat; shock, high temperature. Death 25 hours later.
97556	Collapsed during long distance run; oliguria. Death 34 hours later.
113428	Collapsed during heavy work in heat; oliguria, high blood nonprotein nitrogen. Death 36 hours later.
96554	Comatose after exposure to heat; oliguria, high nonprotein nitrogen. Death 3 days later.
102705	Heat exhaustion; oliguria, albuminuria, high nonprotein nitrogen. Death 6 days later.
89594	Collapse after therapeutic hyperthermia; anuria, high nonprotein nitrogen. Death 6 days later.

The gross and microscopic changes found after death from heat stroke, therapeutic hyperthermia, and heat exhaustion were of the same types as described in the previous groups. Petechial hemorrhages were found in every case. The lungs were intensely hyperemic in 11, moderately hyperemic in one. Pulmonary edema was present in 9, atelectasis in 6, incipient pneumonia in 2. The spleen was engorged in 6. The kidneys were hyperemic in every instance. The myocardium was hyperemic in 7, edematous in 8, and showed degeneration in every case.

Parenchymatous degeneration and necrosis of the liver, kidneys, and heart were present regularly and were more extreme in degree than those seen in any other group of cases. It is known that post-mortem changes develop most rapidly after death from heat stroke; perhaps this is due to the high body temperature which regularly pre-

ceded death. In 5 instances necropsies were performed in 1 to 3 hours after death; the changes mentioned were equally intense in these cases.

It is noteworthy that signs of renal disturbance occur incident to heat stroke. Albuminuria was noted in 4, oliguria in 4, and retention of nitrogenous wastes in 6. In many instances no laboratory studies on the urine and blood had been made. On microscopic examination, casts were found in the renal tubules in 6 of these cases (Figs. 25 and 26).

Subsequent to these studies, an extended survey on the pathology of heat stroke has been published by Malamud, Haymaker, and Custer⁴⁶ based upon material from 125 cases in the Army Institute of Pathology. Hyperthermia was a clinical feature in all but 2 of these. Clinical signs of shock were prominent; the ultimate outcome usually depended upon the degree of shock sustained. Pathologic changes in the central nervous system were conspicuous: progressive degeneration of neurons, congestion, edema, and petechial hemorrhages. The degeneration was attributed to excessive heat, the other changes to shock. "Evidences of acute circulatory failure, such as hemorrhage, edema, and vascular engorgement, were observed in virtually all cases regardless of the duration of illness." Serous effusions were recorded in 33 cases. "In virtually all cases there was intense vascular [pulmonary] congestion." This regularly was accompanied by edema; in no case was the weight of the lungs within normal limits. Some degree of pneumonia was found in 31 cases. Myocardial degeneration was frequent, ranging from irregular patches to large foci of necrosis. The kidneys regularly were hyperemic and above normal weight. The degree of parenchymatous change was less in cases of short duration. "Lower nephron nephrosis" was observed in 19 cases; this was severe in those who lived 35 hours or longer. The livers were congested and above normal weight; central lobular necrosis was seen in 12 of the cases which survived 31 hours or longer.

The recorded data support the belief that secondary shock is an important factor in death from heat stroke. Thermic deaths are accompanied by acute neurologic lesions. The mechanism by which such lesions cause shock has not been shown, but the presence of shock in such cases seems clearly established. The associated anoxia probably causes the parenchymatous degeneration and necrosis seen in the kidneys, liver, and heart. The clinical evidence of cerebral damage, the accompanying high fever, and the pathologic conditions found in the brain and in the viscera indicate that deaths from low atmospheric pressure and from heat stroke have certain features in common.

ABDOMINAL EMERGENCIES

It has been observed that serious abdominal conditions may present the syndrome of shock. Instances are seen in perforations of the viscera, acute pancreatitis, intestinal obstruction as by volvulus or strangulated hernia, mesenteric thrombosis, and others. In several of these, such as acute hemorrhagic pancreatitis, perforation of the gall-bladder or of an ulcer, the onset of shock is immediate. In others, such as intestinal obstruction or peritonitis, the circulatory failure may be delayed somewhat. Ten such cases were surveyed:

A.I.P. Acces-

<i>sion Number</i>	<i>Conditions</i>
101591	Intestinal infarction, shock, anuria. Death 2.5 hours later.
106742	Intestinal obstruction, resection of gangrenous bowel. Death 24 hours later.
108433	Intestinal obstruction, operation for volvulus, anuria, albuminuria.
114688	Intestinal obstruction, operation. Collapse and death on 5th post-operative day.
116017	Intestinal obstruction, colostomy, followed by shock.
117545	Intestinal obstruction, operation. Death 2 days later.
123485	Intussusception, operation, anuria and albuminuria. Death 7 days later.
111691	Acute pancreatitis, oliguria, albuminuria. Collapse and death.
130718	Acute pancreatic necrosis of 2 days' duration, anuria, albuminuria, death.
133910	Acute pancreatitis, high nonprotein nitrogen, hemoconcentration. Death 5 days later.

The findings in these cases were of exactly the same character as in shock from trauma and from other causes. The lungs were intensely congested and edematous in 7, moderately so in 3. Serous effusions were recorded in 7, petechiae in 6. The kidneys were markedly hyperemic in 3, moderately so in 7. The spleen was hyperemic in only 4. Parenchymatous degeneration ranging to necrosis was present in the liver and in the renal tubules in 6; post-mortem changes in 4 made the presence of these changes uncertain. The myocardium showed degenerative changes present in 7, absent in one; in 2 cases sections of heart were lacking.

In 6 cases, surgical relief had been attempted. These might have been included appropriately in the first group. They represent shock resulting from a combination of factors, including anesthesia, surgical trauma, local loss of blood and fluid, and the grave condition of disease which made operation necessary. In the remaining 4 instances, the disease itself led to circulatory failure and death. It is significant that evidences of renal disturbance were seen clinically in several cases; in others no laboratory tests were recorded.

MISCELLANEOUS

Under the heading of miscellaneous are included deaths from various causes including anaphylaxis, allergic reactions, and sudden death from unexplained causes. Sometimes acute infection or poison was shown as the cause of death by post-mortem examinations; such cases are not included in this group. Ten representative instances of sudden or unexplained death were studied.

A.I.P. Access-

<i>sion Number</i>	<i>Conditions</i>
86556	Child died in bed; no previous illness, lymphoid hyperplasia found.
104029	Shock reaction after injection of nupercaine. Death 1 hour later.
105156	Received typhoid and cholera vaccine, collapse. Death 20 minutes later.
109249	Sudden death, cause undetermined.
116783	Typhoid vaccine intravenously, treatment for arthritis. Death 23 hours later.
116840	Received typhus vaccine, no immediate reaction. Died during night.
121120	Shock reaction to diphtheria antitoxin. Death 14 hours later.
128537	Sudden death, cause undetermined.
131480	Sudden death, cause undetermined.
145523	Sudden death, cause undetermined.

In each instance, pulmonary hyperemia and edema were marked; petechiae were present in 9; serous effusions were found in 2. The kidneys were hyperemic in 9, the liver in 7, the spleen in 5. The liver and kidneys showed marked degeneration, ranging to necrosis in 7 cases; post-mortem changes in 3 made degeneration uncertain.

It is known that the visceral findings after anaphylactic death in man and in some animals often are identical with those of secondary shock. Those dying of trivial or insufficient causes often are shown to have a subnormal amount of adrenal cortical tissue. This has been suggested as an explanation for status lymphaticus. The adrenals were hypoplastic in cases 86556, 105156, 109249, and 145523; in 2 other cases no sections of the adrenals were available. Since the adrenal cortical hormone is an important factor in the "alarm reaction" (Selye⁴⁷), deficiency of it may be a factor in deaths without apparent adequate cause.

RENAL PATHOLOGY

It has been observed by many authors that shock from diverse causes was accompanied by an increase in the nonprotein nitrogen of the blood. This was thought by Whipple, Smith, and Belt⁴⁸ to arise from two sources: disintegration of the body's own protein, and deficient elimination of nitrogenous wastes by the kidneys.

Evidence of renal effects accumulated as the phenomena of shock

were studied, until by 1941 their importance appeared so paramount that they were included among characteristic functional disturbances in a definition of shock.²⁶ That evidence is too extensive for detailed review here. It included Bell's⁴⁹ report on "clinical acute nephritis" as distinct from glomerulonephritis, the contributions of Bordley²⁸ and Daniels, Leonard, and Holtzman⁵⁰ on renal effects of transfusions, Lambret and Driessens'⁵¹ studies on physiologic disturbances accompanying surgical shock, Helwig and Schutz'⁵² and Boyce and McFetridge's⁵³ reports on uremia associated with so-called "liver death," Christophe's⁵⁴ observations on "acute nephritis" as a regular feature of burns, both clinical and experimental, Bywaters'⁴⁰ observation on uremia from compression of limbs, and the review of Jeghers and Bakst⁵⁵ on extrarenal uremia.

The reported conditions under which acute uremia developed, independent of glomerulonephritis, included intestinal obstruction, toxic jaundice, "hepatorenal syndrome," extensive surgery, intestinal perforation, poisoning with phenobarbital, cinchophen and other drugs, streptococcal cellulitis and other severe infections, diabetic coma and other metabolic intoxications, anaphylactic reactions, transfusion with incompatible blood, acute pancreatitis, cerebral lesions such as abscess or hemorrhage, sublethal shock from trauma as in the "crush syndrome," and burns. In many instances it was recognized that the renal deficiency was associated with shock. Some found hemoconcentration of value in differentiating this type of uremia from other types.

In the cases summarized here, renal deficiency was prominent when a state of sublethal shock had persisted for 2 days or longer. Instances of this were seen after accidental trauma; gunshot wounds; extensive surgical operations; transfusion reactions; burns; poisoning with arsenicals, mercury, barbiturates and with phosphorus; tularemia, malaria, and other infections; shock from low atmospheric pressure, therapeutic hyperthermia, and from heat stroke, intestinal obstruction, and after miscellaneous causes. In many of these instances, neither transfusion of blood nor chemotherapy, as with sulfonamide drugs, had been employed.

The pathologic features set forth in previous reports do not differ in essential particulars from those described in the groups of cases summarized here. Grossly, the kidneys may be of normal size or moderately enlarged; usually they are soft, indicating edema. The capsule often strips with unusual ease; the stellate veins in the cortical surfaces are engorged. The color varies, depending upon relative degree of congestion and of parenchymatous changes. When congestion pre-

dominates, the color is deep red; degenerative changes produce varying degrees of pallor; cloudy swelling usually is mentioned. Red streaks are seen in the medulla and the cortical markings frequently are obscured. Sometimes petechiae are described in the parenchyma or in the pelvic lining.

The microscopic picture shows varying degrees of changes within a regular pattern. Usually hyperemia of the glomerular tufts and of the intertubular capillaries and venules is conspicuous. Sometimes capillary hemorrhages are seen. Red streaks in the medulla result from patchy dilatation of groups of venules which parallel the straight tubules. The epithelium of the convoluted tubules shows varying degrees of acute degeneration. The cytoplasm is granular; more marked degeneration is indicated by vacuolation of the cytoplasm or disintegration of the cells. The nuclei may be hyperchromic, hypochromic, or karyolytic.

Hyaline droplets in the cytoplasm are seen often when degeneration is marked; the lumina may contain globules or masses of this deeply acidophilic material when the cells containing it have disintegrated. Because of their dense staining, these may be mistaken for red blood cells or their pigment. Sometimes these changes are more marked in the upper segment, sometimes in the lower, but usually all portions of the convoluted tubules are affected. Plugs of *débris* often occur in the lumina of the lower segments as such *débris*, originating in the upper portion of the nephron, collects in the narrow lumina of the lower portion. Hyaline, granular, and sometimes pigmented casts, *débris*, erythrocytes, and the nuclei of the degenerated epithelial cells are usually found in the collecting tubules. Edema is a varying feature usually more marked in the medullary than in the cortical zones.

Two recent reports have dealt with renal changes associated with shock. Herbut⁵⁶ confirmed the syndrome of uremia noted by others, consisting of oliguria, albuminuria, casts, erythrocytes and *débris* in the urine, and progressive azotemia. These features developed incident to acute or to sublethal shock from diverse causes. Cases were described resulting from transfusion reactions, bile peritonitis, ulcerative enteritis, abscess, burns, extensive necrosis of neoplasm, obstructive jaundice, sulfonamide therapy, mercuric and arsenical poisoning. The gross features of the kidneys were as described above. The microscopic characteristics were hyperemia and edema of the glomeruli and interstitial tissue; severe degeneration ranging to necrosis of the tubular epithelium, most marked in *proximal* portions; granular eosino-

phile material in the lumina of both proximal and distal portions; and various types of casts in the distal and collecting tubules.

Lucké⁵⁷ reported on "lower nephron nephrosis," based upon records and material from numerous cases in the Army Institute of Pathology. These represented battle wounds, crushing injuries, abdominal operations, burns, transfusion reactions, sulfonamide intoxication (combined with infections, trauma, etc.), heat stroke, malaria, various poisons, hemolytic anemia, and miscellaneous conditions such as eclampsia, pancreatitis, and shock from various causes. Shock and vomiting were the two conditions most commonly associated. The clinical manifestations were oliguria, dark or bloody urine containing heme pigment, albumin and casts, progressive azotemia, hypertension, edema, and uremia. The gross features of the kidneys were essentially as given above. Microscopically, acute degeneration ranging to necrosis was found regularly. This involved chiefly the *lower* segments of the convoluted tubules. Protein material was present in the capsular spaces. The tubular lumina contained pigmented and nonpigmented casts and cellular débris.

These changes were as observed by others except for one particular: Lucké⁵⁷ found degeneration and necrosis sharply limited to the lower portion of the nephron. Others have described such changes in similar cases as involving all parts of the convoluted tubules. My own observations coincide with the latter findings (Figs. 4, 7, 18, 20, 24, and 25).

SUMMARY

In the cases studied, the pattern of visceral changes characteristic of secondary shock was found after death from various forms of acute disease. These included the combined effects of trauma, hemorrhages, infection, anesthesia, transfusion, and surgery; burns, poisoning with various drugs and chemicals, severe infections, anoxia from sundry causes, low atmospheric pressure, heat stroke, abdominal emergencies such as intestinal obstruction and pancreatitis, and sudden death from anaphylaxis and other causes.

The pattern of changes indicative of endothelial damage included dilatation and engorgement of capillaries and venules in the thoracic, abdominal, and cranial viscera, petechiae in serous and mucous surfaces and within parenchymatous tissues, and edema of soft viscera. Two modes of origin are suggested for the acute degeneration ranging to necrosis which was seen in the kidneys, liver, and myocardium: (1) The same injurious agents which damage endothelium and cause

capillary dilatation and permeability likewise may cause parenchymatous degeneration and necrosis; this is seen in the effects of various poisons and bacterial products. (2) The same effects resulted from uncomplicated anoxia, indicating that endothelial, renal, hepatic, and myocardial cells are delicately susceptible to lack of oxygen. It seems probable that the parenchymatous changes, found after death from shock, result from a combination of these two mechanisms.

Pulmonary hyperemia and edema occurred in a high percentage of cases. When death was delayed 48 hours or longer, the development of secondary or terminal pneumonia often occurred. Lungs in which the circulation is impaired and the spaces filled with albuminous fluid will almost certainly develop secondary pneumonia if neither death nor recovery occurs soon.²⁴ This was exemplified in numerous instances when shock persisted for several days in a sublethal degree.

Some degree of atelectasis was found in almost one-half of the cases. No previous comment has been made on this feature; its origin and relationship to the mechanism of shock are not apparent. Hemorrhagic infarcts, suggesting embolism, were found in several cases.

Renal functional deficiency was a prominent feature of sublethal shock lasting several days. This was manifested clinically by oliguria, albuminuria, hematuria, and by progressive retention of nitrogenous wastes sometimes terminating in uremia. The pathologic renal features included hyperemia and edema; parenchymatous degeneration and necrosis of tubular epithelium, with desquamation into the lumina; débris; hyaline, granular, and sometimes pigmented casts. These features correspond to the picture of *acute tubular* or *parenchymatous* nephritis described by internists and pathologists of the preceding generation.

Hepatic degeneration and necrosis were regular features, but their degree and distribution were inconstant. Degeneration tended to be diffuse and the degree varied from granular cytoplasm and vesicular nuclei to necrosis. Absence of nuclei, pyknosis, and disintegrating cells were the criteria for necrosis. Frequently this involved only scattered groups of cells; when the groups were larger, focal necrosis was seen. Occasionally, as after burns, heat stroke, or death from low atmospheric pressure, extensive necrosis involved the centers of the lobules and resembled that produced by poisons.

Degeneration of the myocardium was less constant than that of the kidneys and liver; its degree varied. Often the cross striations were obliterated, the substance of the fibers was granular or lumpy rather than of uniform density, and the nuclei were swollen or distorted.

Apparent liquefaction of the fibers, basophilic staining reaction, or marked transverse fragmentation indicated severe degeneration.

Splenic changes were not consistent; in some instances the spleen was enlarged and engorged, in others it was flabby, contracted, and ischemic. In a number of sections it was noted that the lymphoid follicles were small, inconspicuous, and contained few lymphoid cells. This apparently did not pertain to any one type of causative condition; it was observed in several of the groups. There was no evidence to indicate whether its origin is related to shock. The histologic preparations did not always include lymph nodes; when present they were regularly edematous and occasionally hyperemic.

Hyperemia, edema, and petechiae often were found in the mucosa of the stomach and small bowel. This feature was not regular. Petechial hemorrhages in mucous and serous surfaces and in the substance of organs were present in the majority of cases. Their highest frequency was after poisoning, burns, anoxia, and after heat stroke. This indicates that lack of oxygen is an important factor in endothelial damage.

Sections of adrenal were available in about one-half of the cases. In the majority of these the cells of the zona fasciculata appeared abnormally vacuolated. In some, the cells were disintegrated and in a few instances there was focal necrosis. These observations are in accord with those of Selye⁴⁷ who emphasized depletion of the adrenal cortical cells as a feature of shock. In a few cases of fulminating meningococcal meningitis, the adrenals were diffusely hemorrhagic and almost totally necrotic. This was not seen in any instance of shock from other causes.

Other ductless glands were not regularly included among the sections available. In several instances the pituitary body was markedly hyperemic and the cells of the anterior lobe appeared degenerated. Occasionally the thyroid and thymus were hyperemic.

This study corroborates the occurrence of secondary shock in other conditions than severe trauma, burns, and after extensive surgery. It develops in sundry conditions which may cause either capillary atony or anoxia. The resulting circulatory effects in the viscera indicate widespread capillary damage. The changes varied somewhat in degree but the pattern was consistent.

The parenchymatous effects may be ascribed in part to the injurious agent itself, in part to anoxia. The severe effects seen regularly in the renal tubular epithelium probably are related to the anuria and other evidences of renal functional deficiency which accompany shock.

It appears that secondary shock, like other conditions of disease, is accompanied by distinctive morphologic changes which are related to its mechanism of origin and to the associated functional disturbances.

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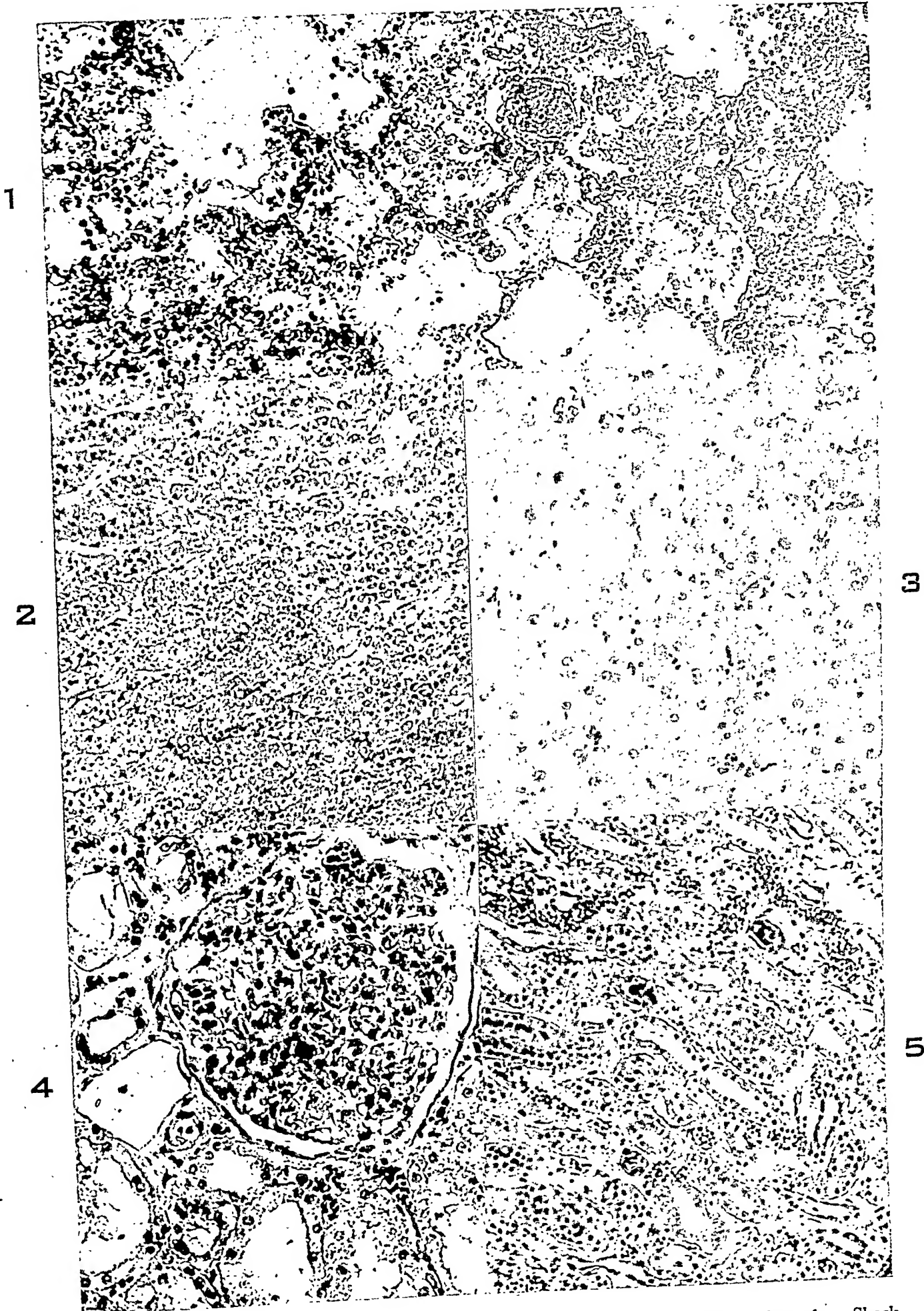
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DESCRIPTION OF PLATES

PLATE 52

- FIG. 1. Army Institute of Pathology accession no. 114945, multiple gunshot wounds. Lung showing intense capillovenous hyperemia, edema, capillary hemorrhages, and slight leukocytic infiltration. $\times 160$. (A.I.P. negative no. 86843.)
- FIG. 2. From the same case as Figure 1. Adrenal cortex showing focal necrosis. $\times 145$. (Neg. 89858.)
- FIG. 3. A.I.P. acc. 102060, shock following surgery, death after 4 days. Liver showing acute degeneration and necrosis. $\times 220$. (Neg. 89849.)
- FIG. 4. From the same case as Figure 3. Renal cortex showing albuminous matter in the capsular space, degeneration and necrosis of tubular epithelium affecting both upper and lower segments, and debris in the lumina. $\times 250$. (Neg. 86826.)
- FIG. 5. Renal medulla from the same section as Figure 4. Of note are masses of desquamated renal epithelium filling the lumina of the collecting tubules. Many contain erythrocytes. $\times 160$. (Neg. 86836.)



Pathology of Secondary Shock

PLATE 53

- FIG. 6. A.I.P. acc. 11,4072, death 8 hours after burns of the skin. Lung showing intense hyperemia, edema, and capillary hemorrhages. $\times 145$. (Neg. 68819.)
- FIG. 7. A.I.P. acc. 118506, death 8 days after burns, post-mortem interval of 1 hour. Renal cortex showing acute degeneration, necrosis of individual cells, casts and debris in lumina, and albuminous matter in capsular space. $\times 230$. (Neg. 86813.)
- FIG. 8. Renal medulla from the same section as Figure 7. Of note are hyperemia, casts, and debris in the lumina. $\times 230$. (Neg. 86821.)
- FIG. 9. Liver from the same case as Figure 8. Marked acute degeneration and necrosis. $\times 280$. (Neg. 89850.)
- FIG. 10. From the same case as Figures 3 to 5. Lung showing hyperemia, edema, and beginning pneumonia. $\times 160$. (Neg. 86824.)

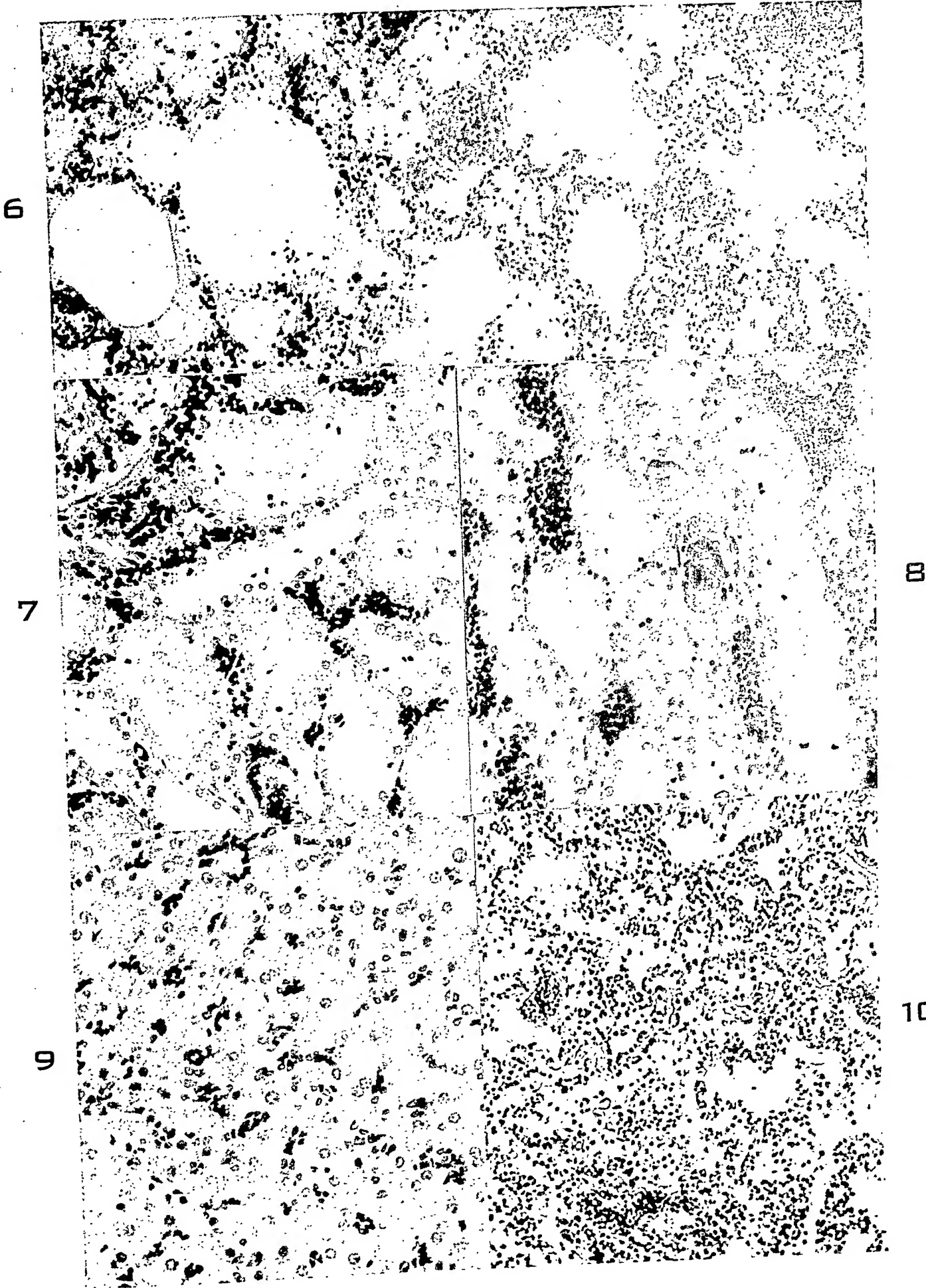


PLATE 54

- FIG. 11. A.I.P. acc. 111925, shock reaction to mapharsen. Lung showing hyperemia, edema, and early pneumonia. $\times 130$. (Neg. 89855.)
- FIG. 12. From the same case as Figure 11. Renal cortex, showing degeneration and necrosis of the tubular epithelium, casts, hyperemia, and edema. $\times 145$. (Neg. 89856.)
- FIG. 13. From the same case as Figures 11 and 12. Renal medulla, showing granular and pigmented casts and erythrocytes in the lumina. Edema and hyperemia are seen also. $\times 175$. (Neg. 86829.)
- FIG. 14. A.I.P. acc. 116989, poisoning with phenobarbital. Myocardium showing degeneration and fragmentation. $\times 160$. (Neg. 89864.)
- FIG. 15. A.I.P. acc. 118865, fulminating meningitis. Lung showing hyperemia, edema, and slight atelectasis. $\times 120$. (Neg. 89863.)

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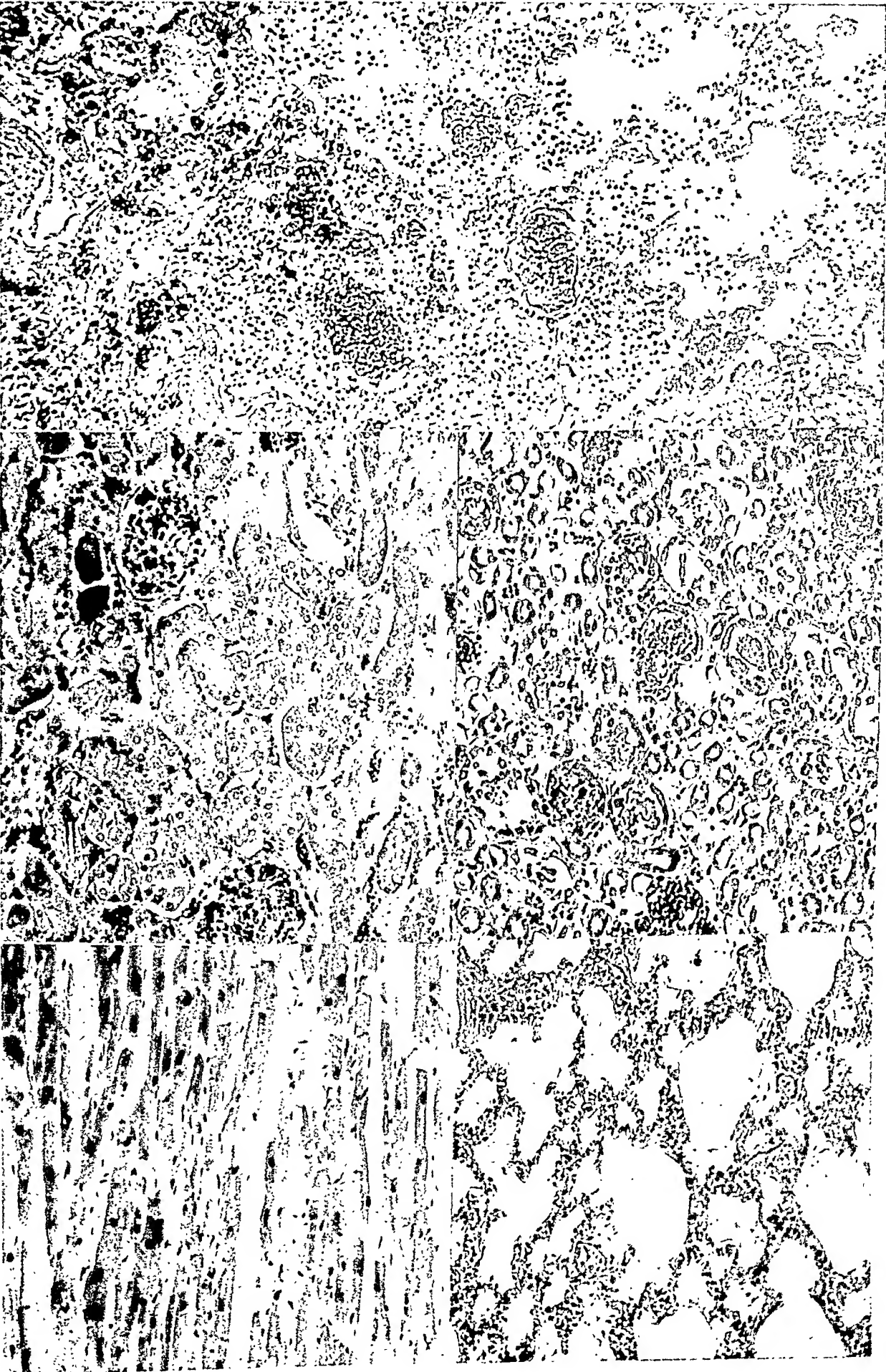
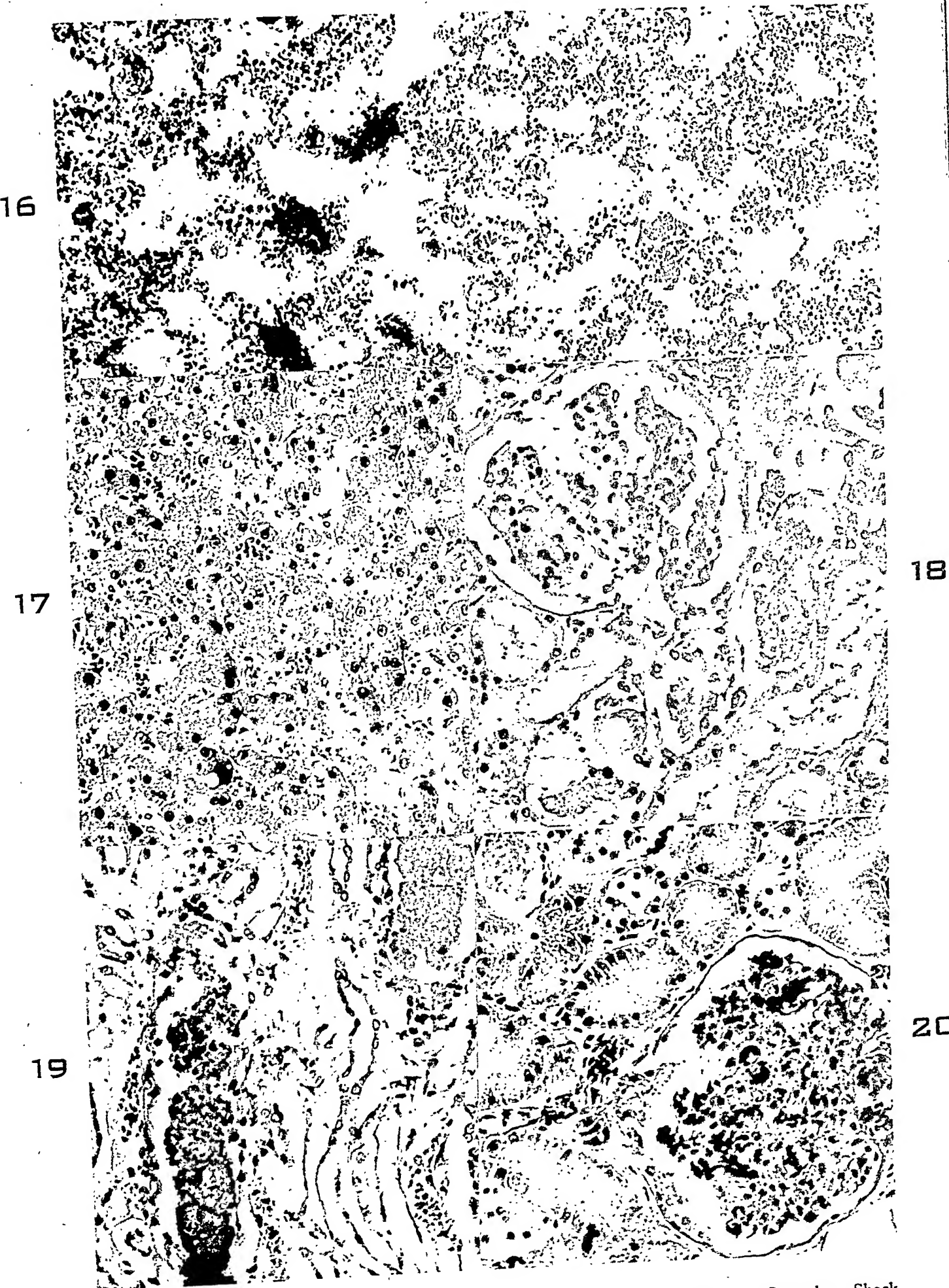


PLATE 55

- FIG. 16. A.I.P. acc. 100893, shock from low atmospheric pressure in experimental test. Intense hyperemia, edema, and slight leukocytic infiltration are shown in this lung. $\times 145$. (Neg. 86838.)
- FIG. 17. From the same case as Figure 16. Liver showing acute degeneration, necrosis of individual cells, and edema. $\times 230$. (Neg. 86834.)
- FIG. 18. From the same case as Figures 16 and 17. Renal cortex showing low cuboidal epithelium, debris and casts in wide lumina, degeneration and necrosis of epithelium, and albuminous matter in capsular space. $\times 250$. (Neg. 86835.)
- FIG. 19. From the same case as Figures 16 to 18. Renal medulla, same section as Figure 18. Of note are the granular, amorphous, pigmented, and epithelial casts. No transfusion of blood and no drug therapy had been given. $\times 250$. (Neg. 86817.)
- FIG. 20. A.I.P. acc. 127451, shock from high-altitude aviation. Renal cortex showing marked degeneration and necrosis, desquamation, debris in lumina, and in the capsular space. $\times 280$. (Neg. 86812.)



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PLATE 56

FIG. 21. A.I.P. acc. 128485, intestinal obstruction and surgery. Lung showing hyperemia and edema. $\times 175$. (Neg. S6840.)

FIG. 22. A.I.P. acc. 128537, sudden death, cause undetermined. Myocardium showing marked degeneration, fragmentation, necrosis, and slight edema. $\times 180$. (Neg. S6833.)

FIG. 23. A.I.P. acc. S3172, death from lack of oxygen. Liver showing acute degeneration, early necrosis, and marked edema. $\times 230$. (Neg. S6810.)

FIG. 24. A.I.P. acc. 111237, lack of oxygen in high-altitude aviation. Renal cortex showing acute degeneration of epithelium, many pyknotic nuclei, and albuminous matter in the capsular space. $\times 300$. (Neg. S6831.)

FIG. 25. A.I.P. acc. 113428, heat stroke fatal in 36 hours. Renal cortex showing extensive necrosis, casts, and debris in the lumina. $\times 230$. (Neg. S6825.)

FIG. 26. Same section as Figure 25. Renal medulla. Of note are the granular, hyaline, amorphous, and pigmented casts. This patient had received no transfusion of blood nor drug therapy. $\times 230$. (Neg. S6822.)

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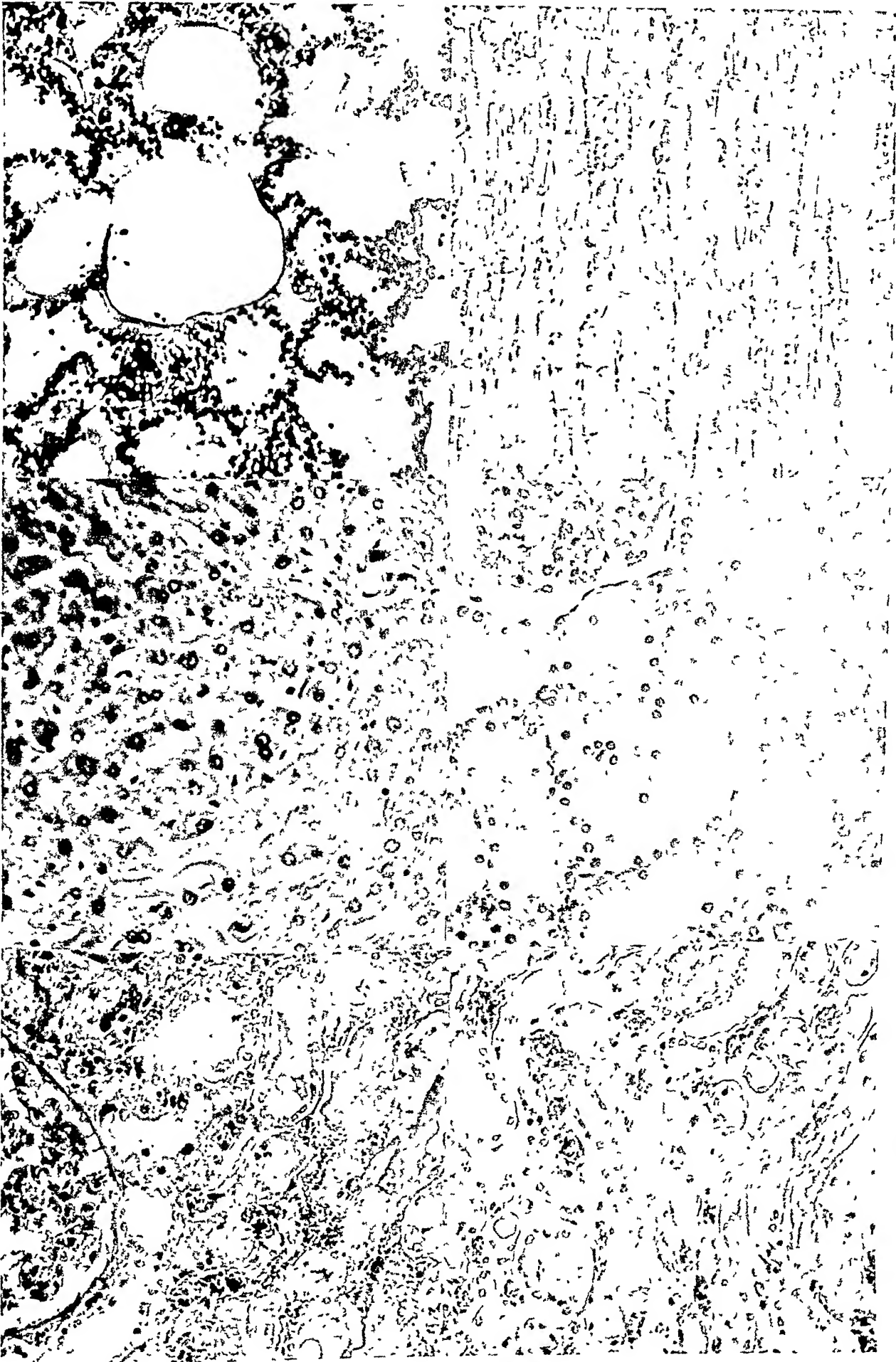
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Moon

Pathology of Secondary Shock

STUDIES ON THE MECHANISM OF ACTION OF THE NITROGEN AND SULFUR MUSTARDS IN VIVO *

DAVID A. KARNOFSKY, M.D., IRVING GRAEF, M.D., AND HOMER W. SMITH, Sc.D.

(From the Departments of Physiology and Pathology, New York University
College of Medicine, New York 16, N.Y.)

The biologic actions of the sulfur and nitrogen mustards have been reviewed recently,¹ and detailed descriptions of the toxicologic and pathologic effects of these compounds have been submitted for publication.^{2,3} At LD₅₀ doses, via any route of administration, these compounds produce a characteristic toxicologic *systemic* effect consisting of anorexia, weight loss, diarrhea, and leukopenia, terminating in death 3 to 5 days after injection. Clinical and pathologic examinations show that lymphopenia appears within 6 to 12 hours after injection and is associated with lymphocytic destruction and involution of the lymphatic tissue, thymus, and spleen. This is followed by progressive decrease in granulocytes to the low level of 200 to 300 cells per cmm. within 3 days after injection, associated with aplasia of the bone marrow; and diarrhea beginning 2 days after injection, associated with demonstrable desquamative degenerative changes in the intestinal mucosa.³

This report consists of a series of experiments designed to elucidate the mechanism whereby these effects are produced.

THE "ALARM REACTION" AND ITS RELATION TO THE LYMPHOCYTO-TOXIC ACTION OF NITROGEN MUSTARD

The systemic intoxication produced by the mustard compounds in rats resembles in many respects a pattern of organic changes that has been termed by Selye^{4,5} the "alarm reaction."[†] This pattern is alleged to follow the subjection of normal rats to a wide variety of unrelated, sublethal injurious procedures, *e.g.*, cold, traumatic injury, excessive muscular exercise, spinal shock, acute infection, and injections of formaldehyde, morphine, atropine, and adrenalin.⁵ According to Selye's description, this reaction consists of "*a rapid decrease in the size of the thymus, spleen, lymph glands and liver; disappearance of fat tissue; edema formation, especially in the thymus and loose retroperi-*

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† "An alarm reaction develops after the administration of any drug administered in sublethal doses, unless the specific pharmacological actions of the drug exert such a violent selective action on vital centers (heart, respiratory center, etc.) that death ensues as a result of this selective action before any marked general damage occurs." Selye.⁵

toneal connective tissue; accumulation of pleural and peritoneal transudate; *loss of muscular tone*; fall of body temperature; *formation of acute erosions in the digestive tract*, particularly in the stomach, *small intestine* and appendix; loss of cortical lipoids and chromaffin substance from the adrenals; and sometimes hyperemia of the skin, exophthalmos, increased lachrymation and salivation. In very severe cases, focal necrosis of the liver and dense clouding of the crystalline lens may be observed." (The italics are ours and indicate features which are common in animals intoxicated with the mustard compounds.) Marked leukocytosis with lymphopenia usually occurs within 48 hours with the "alarm reaction," but leukopenia develops if the treatment is severe.⁶

Microscopically, the lymphoid tissue in the "alarm reaction" presents a picture of massive lymphocytic necrosis and fragmentation with contraction of the organ and increase in reticular elements and frequent hemorrhages.⁷ The adrenal cortex becomes hyperplastic with a loss of lipid granules, while the medulla loses its chromaffin granules and vacuoles appear in the periphery of its cells; if the "alarm reaction" is severe the cells undergo necrosis. Other changes occur in the pancreas, the gastric and intestinal mucosa, and the liver.

Selye postulated that the "alarm reaction" is due to a common substance released in the body by all varieties of noxious stimuli. In normal rats this substance induces adrenal hypertrophy and hypersecretion, which in turn causes involution of the lymphatic tissue, for in the absence of the adrenal glands the "alarm reaction" does not induce lymphatic atrophy.⁵ Selye stated that "So far, the only substances with which we have been able to cause thymus involution in the adrenalectomized rat are cortical extract and estrone."

It is to be assumed under Selye's hypothesis that the mustard compounds, because of their severe and prolonged intoxicating effect, must invoke some degree of the "alarm reaction." It is necessary to demonstrate, however, that the specific pathologic effects attributable to the mustard compounds are not indirectly due to the "alarm reaction." In the case of the lymphatic tissue, this was shown in adrenalectomized rats as follows.

THE EFFECT OF METHYL-BIS (β -CHLOROETHYL) AMINE HYDROCHLORIDE (HN₂·HCL)* IN ADRENALECTOMIZED RATS

Before a definitive experiment could be performed, the toxicity of HN₂·HCl in adrenalectomized rats was determined. Such rats, ade-

* HN₂ is the official designation of this nitrogen mustard compound; HN₂·HCl is the hydrochloride salt.

quately maintained on salt, are unduly sensitive to HN_2 intoxication both in regard to dosage and to the acceleration of intoxication; 10 mg. per kg. of the HCl salt given subcutaneously are fatal in 3 to 6 hours after injection, whereas most normal rats survive for 72 hours; 3 mg. per kg. cause death in 48 to 60 hours, instead of 78 to 100 hours for the controls; and 1 mg. per kg. ($\frac{1}{2}$ LD_{50}) is fatal in 3 to 4 days whereas controls survive such a dose without difficulty. A dose of 3 mg. per kg. was used in the experiment reported.

Methods

Fifty-six adult female rats, weighing from 150 to 210 gm., were offered 1 per cent saline solution in tap water beginning 24 hours before operation and during the course of the experiment. Four groups were employed.

Group I. (8 Sham-operated Control Rats.) The rats were placed on limited rations so that they suffered a slight progressive weight loss. It is difficult to prepare a control group whose food intake and weight loss will parallel that seen after HN_2 since changes in fluid balance and gastric distention with food complicate the picture in the intoxicated rat. It was felt, however, that animals on decreased food intake for a short period would furnish a more adequate control than normal well fed rats.

Group II. (10 Adrenalectomized Rats.) These were fed *ad libitum*.

Group III. (18 Sham-operated Rats Receiving 3 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ Subcutaneously 24 Hours after Operation.) These animals were fed *ad libitum*.

Group IV. (20 Adrenalectomized Rats Receiving 3 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ Subcutaneously 24 Hours after Operation.) These animals were fed *ad libitum*.

Operations were performed under ether anesthesia. Twenty-four hours after operation 3 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ in 0.8 cc. of saline solution were given to each animal in the appropriate groups. This represents an LD_{99} dose in normal animals, the LD_{50} being 1.9 mg. per kg. The rats were weighed daily. Sacrifices were performed by exsanguination from the abdominal aorta under ether anesthesia. Blood counts and smears were taken from the freely flowing aortic blood at autopsy. Organs were removed, blotted to free them of blood, and weighed to the nearest milligram on a Roller-Smith torsion balance. The lungs were weighed in tared beakers and then dried for 18 hours at 88 to 90° C. The data on the weight of the organs are insufficient to justify corrections for body weight or statistical analysis; only the averages and the extreme range of weights for each organ are given.

TABLE I
Effects of 3 mg. per kg. of HN₂-HCl Methyl-bis (β -chloroethyl) Amine Hydrochloride on the Normal and Adrenalectomized Rat; Body and Organ Weights at Time of Sacrifice

	Controls		Injected with 3 mg. per kg. of HN ₂ -HCl			
	Group I Sham-operated, diet restricted	Group II Adrenalectomized	Group III Sham-operated		Group IV Adrenalectomized	
			Time of sacrifice after injection, in hours			
			24	48	72	24
No. of rats	8	10	5	4	8	5
Pre-treatment wt. (gm.)	193 (182-200)	182 (170-195)	184 (170-200)	184 (172-200)	195 (185-208)	190 (175-200)
Aver. wt. at sacrifice (gm.)	178 (169-192)	181 (168-208)	172 (164-184)	163 (150-178)	162 (150-185)	171 (142-192)
Aver. wt. of liver (gm.)	5.90 (5.2-6.3)	6.45 (5.4-8.4)	7.35 (7.1-7.5)	6.30 (5.2-7.5)	5.70 (5.2-6.4)	6.25 (4.9-7.0)
Aver. wt. of spleen (mg.)	865 (660-1,260)	1,140 (719-1,948)	541 (375-804)	446 (337-536)	333 (168-285)	452 (357-566)
Aver. wt. of adrenals (mg.)	50 (42-55)		61 (48-79)	58 (51-72)	79 (62-94)	
Aver. wt. of thymus (mg.)	155 (110-206)	280 (172-392)	102 (73-139)	79 (61-100)	39 (33-61)	94 (65-126)
Aver. wt. of cervical lymph node (mg.)	73 (45-100)	79 (54-117)	55 (53-56)	48 (34-70)	23 (15-30)	71 (54-90)
Aver. wt. of heart (mg.)	584 (517-660)	575 (512-648)	584 (520-726)	525 (451-587)	585 (577-634)	463 (464-634)
Aver. wet wt. of lungs (gm.)	1.44 (1.25-1.71)	1.45 (1.23-1.79)	1.53 (1.07-2.39)	1.09 (0.91-1.22)	1.17 (1.01-1.34)	1.40 (1.32-1.51)
Aver. dry wt. of lungs (mg.)	306 (276-351)	294 (259-346)	343 (252-504)	284 (223-331)	314 (276-341)	305 (262-362)
Aver. per cent of water in lungs	78.7 (77.9-79.3)	79.7 (79.0-80.7)	77.2 (75.2-78.9)	73.8 (70.4-76.3)	73.3 (72.7-73.9)	78.3 (76.1-79.4)
Aver. total white cell count	12,260	13,000	3,240	450	300	1,070

The results are given in Table I and the several observations of interest are discussed under the appropriate group.

Results

Group I. The sham-operated control group on a restricted diet lost about 8 per cent of their body weight, representing a moderate degree of inanition. Necropsy findings were not remarkable. Organ weights are tabulated in Table I.

Group II. Two of the 10 adrenalectomized rats were sacrificed at 1 day, 2 at 2 days, and 6 at 8 days after operation; the organ weights of these animals, which were not appreciably different, are averaged together in Table I. The rats seemed well during the experiment. On 1 per cent saline drinking water, adrenalectomized animals gained 10 to 15 gm. in weight immediately after operation because of retention of fluid, which they then lost gradually. At sacrifice the thymuses and spleens were larger than those of Group II (controls, diet restricted). The lungs showed a slight increase in water. The other organs were not significantly altered, and no adrenal tissue was found. Leukocyte counts were normal, and the blood smears, while suggesting an increase in lymphocytes, were too few for valid interpretation.

Group III. Three mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ given subcutaneously produced severe effects in normal rats. At 24 hours the rats had lost a little weight and seemed moderately depressed, at 48 hours slight diarrhea appeared, and between 48 and 72 hours marked symptomatic changes developed. The rats appeared cold, were hunched-up, and huddled together. An almost continuous watery diarrhea, which contained considerable mucus, completely soaked the anal region and lower abdomen, and weight loss was marked. Neurologic changes were not noted, but the rats became more depressed and inactive. Those not sacrificed died between 80 and 102 hours.

Five rats were sacrificed at 24 hours, 4 at 48 hours, and 8 at 72 hours. The organ weights were averaged separately for each of these groups. At 24 hours the stomach contained a moderate amount of food, and in the small intestine small amounts of air and mucus were found. One animal had a small hemorrhagic patch in the ileum, and the mesenteric nodes were reddened. The thymus, spleen, and lymph nodes were decreased in size, and the adrenals appeared to be larger than is normal. The lungs were of normal weight but the fluid content appeared to be slightly reduced. The fall in white blood cell count was already marked and, although the lymphocytes were most severely depressed, the granulocytes had begun to fall also. At 48 hours the stomach was moderately filled with food, and the fluid in the small intestine appeared increased. The lymph nodes were hemorrhagic and those in the ileoce-

cal region were markedly so. The spleen and thymus were about half of the control weights and the lymph nodes were reduced in size. The suggestive increase in adrenal weight was still present. A remarkable finding was the sharp fall in the wet weight of the lungs, with retention of normal dry weight, indicating approximately 30 per cent decrease in the water content of the lungs. The average total leukocyte count had fallen to 450. At 72 hours, the stomach was distended with considerable food and fluid, and the intestines and cecum contained excess amounts of fluid, often including mucus. The colon was usually empty, but contained almost pure mucus in one animal. Small petechial hemorrhages often were found in the mesentery and hyperemic areas were present in the duodenum and lower ileum, the latter in one rat containing several deep erosions of the mucosa. Peyer's patches were hemorrhagic and the regional lymph nodes were small and red. The spleen, thymus, and lymph nodes were reduced to about 25 to 35 per cent of their control size. The increase in adrenal weight was unquestionable, and the livers were decreased in weight. The water content of the lungs remained decreased, being about 25 per cent of that of control group II.

Group IV. Rats receiving 3 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ 24 hours after adrenalectomy showed a rapid acceleration in the development of toxic symptoms. Two animals died within 7 hours. At 24 hours the remaining rats appeared very weak, depressed, and had mild watery diarrhea; at 48 hours they were either dead or moribund; those remaining were huddled together, cold, moved with difficulty, and all died within 60 hours after injection. Diarrhea was moderate and weight loss was not marked. Four rats were sacrificed at 24, and 5 rats at 48 hours. At 24 hours the stomach was filled largely with fluid, the duodenum was slightly congested, and the small intestine greatly distended with clear fluid. The entire animal seemed excessively moist, and there was free peritoneal and thoracic fluid. The spleen, thymus, and lymph nodes were reduced in size, as markedly as those seen in the control animals treated with $\text{HN}_2\cdot\text{HCl}$ (group III). The lymph nodes seemed slightly hemorrhagic. The water content of the lungs was slightly greater than is normal. The average total leukocyte count was 1,600. At 48 hours the rats were moribund when sacrificed. Their stomachs and small intestines were greatly distended with fluid. The walls of the duodenum were reddened and frequently hemorrhagic. The mesentery had a few small petechial hemorrhages and the fat had a peculiar white, granular appearance. The decrease in weight of the thymus and spleen was not much greater than that at 24 hours, and the weights of these organs did not differ significantly from those in group III. The water content of the lungs was only slightly decreased.

The average leukocyte count was 1,070 with 80 per cent lymphocytes; this total count was suggestively higher than that seen in group III.

Microscopic Observations

In the adrenalectomized rats injected with 3 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$, some intact lymphocytes could be found in the thymus, and generally the lymphatic tissue, although containing a diminished number of lymphocytes, appeared to be less severely affected than that of normal rats given the same dose. In another experiment, adrenalectomized rats, receiving 10 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ and sacrificed 3 to 6 hours after injection when they appeared moribund, showed severe lymphocytic fragmentation and chromatin debris in the thymus and lymph nodes, but again these effects seemed somewhat less than in the controls treated with the same dose. It should be noted that although 1 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ given subcutaneously, a sublethal dose in normal rats, is fatal to adrenalectomized rats, the histologic evidence of injury to the lymphoid tissue, bone marrow, and gastrointestinal tract is no more marked in the adrenalectomized rats than in the controls.

These experiments demonstrate that methyl-*bis* (β -chloroethyl) amine hydrochloride can induce lymphocytic destruction and involution of the thymus, spleen, and lymph nodes in the absence of the adrenal gland. Quantitatively, however, the lymphocytotoxic effect seems to be somewhat less severe in adrenalectomized rats than in normal ones injected with the same dose. The involution of the lymphatic tissue, therefore, results from a direct action of the nitrogen mustard, or is mediated by a different mechanism than that occurring in the "alarm reaction." Since, by definition,⁵ the "alarm reaction" is involved in nitrogen mustard intoxication, it is possible that this mechanism may contribute to a slight extent to the lymphatic involution seen in rats intoxicated with $\text{HN}_2\cdot\text{HCl}$. LeBlond and Segal⁸ have been able to induce involution of the thymus in adrenalectomized rats by x-rays, although these effects were quantitatively less severe than those seen in normal rats. The nitrogen mustards seem to produce a somewhat similar effect.

DEMONSTRATION OF THE DIRECT ACTION OF $\text{HN}_2\cdot\text{HCl}$ INJECTED INTRAVENOUSLY, ON THE INTESTINAL TRACT *

It is shown in the following experiment that the intestinal injury induced by $\text{HN}_2\cdot\text{HCl}$ is due to a *direct* and rapidly completed action

* It had previously been shown in this laboratory that exclusion of the biliary secretion in rats did not prevent HN_2 from inducing characteristic intestinal injury. Since one could have anticipated this result from the experiment cited above, the biliary exclusion experiment is not described in detail.

of this compound on the intestinal tract. This demonstration was accomplished by temporarily occluding the circulation to the intestinal tract during varying, short periods of time after the intravenous injection of the compound, and then observing the nature of the injury produced.

Rats were anesthetized with ether, the abdomen opened, and most of the small intestine exteriorized and placed on cotton soaked with saline solution. Rubber-sheathed hemostats were placed about 5 cm. distal to the pylorus and 1 cm. proximal to the ileocecal valve, and a third hemostat was placed across the root of the mesentery. By this ring of hemostats, circulation to the major portion of the small intestine was occluded for 15 minutes. Control rats subjected to this procedure suffered no ill effects, and gave no evidence of significant histologic damage to the intestinal mucosa.

Following the application of the clamps, 2 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ (1.6 LD_{50}) was injected into the vena cava, and 15 minutes later the clamps were removed. The rats were sacrificed at 60 and 72 hours. Grossly and microscopically the small portion of the duodenum and ileum outside the occluding clamps showed the characteristic injury due to the nitrogen mustards,³ whereas the remainder of the small intestine, which had been surrounded by the clamps, was normal. The toxicity of $\text{HN}_2\cdot\text{HCl}$, however, was not appreciably altered by this procedure.

This experiment clearly demonstrated that $\text{HN}_2\cdot\text{HCl}$ had a rapidly completed, and presumably direct, action on the mucosa of the small intestine.

DEMONSTRATION OF THE DIRECT ACTION OF $\text{HN}_2\cdot\text{HCl}$ INJECTED INTRAVENOUSLY, ON THE BONE MARROW OF THE RAT AND RABBIT

It is shown in the following experiments that $\text{HN}_2\cdot\text{HCl}$ has a direct and rapidly completed action on the bone marrow. This demonstration was accomplished by temporarily occluding the circulation to the hind legs during and for varying short periods after the intravenous injection of $\text{HN}_2\cdot\text{HCl}$.

Rats were anesthetized with ether, the abdomen opened, and an arterial clamp placed on the abdominal aorta and inferior vena cava just below the renal vessels. Two mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ (1.6 LD_{50}) were then injected into the vena cava just above the clamp, and the clamp removed after intervals varying from 5 to 15 minutes. Operated controls carried the clamp for 15 minutes without ill effects and with no changes in the bone marrow. The rats were sacrificed 72 and 96 hours after injection.

At the time of sacrifice, the control rats injected with $\text{HN}_2\cdot\text{HCl}$ had

extreme leukopenia, the leukocyte count being in the range of 50 to 250 per cmm. with cells usually too few to make a differential count significant. Microscopic sections showed the femoral, vertebral, sternal, and humeral marrow to be aplastic. The rats subjected to temporary vascular occlusion had leukocyte counts averaging 1700 per cmm. at 72 hours and 4,000 per cmm. at 96 hours after injection, with the differential count consisting predominantly of granulocytes. Histologic study (Figs. 1 and 2) showed aplastic sternal and humeral marrow, whereas the femoral marrow was extremely cellular and especially rich in myeloid elements. The marrow in the lumbar vertebrae below the level of the occluded aorta was very cellular, whereas that from the upper thoracic vertebrae was aplastic.

Rabbits were anesthetized with ether and, after suitable preparation of the skin, the aorta and vena cava were exposed through a left lateral abdominal incision. A rubber-sheathed hemostat was clamped on the two vessels during and for times varying for 2 to 15 minutes after the injection of 2 to 3 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ (around the LD_{50}) into an ear vein.* Several complications may result from this procedure. If the clamp is left on too long (15 minutes or longer, usually), a gradual paralysis of the hind legs may occur, presumably due to injury in the spinal cord, since the legs do not become edematous, cold, or gangrenous. A dose of 3 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ in the clamped animal frequently produces various neurologic manifestations such as tremors, ataxia, and convulsions, which in some instances seemed to lead to death within a period seen only when two to three times this dose is given to the normal rabbit. This would suggest, incidentally, that the lower part of the body accounts for a considerable amount of the chemical when it is injected into the intact animal. Because of these effects, a dose of 2 mg. per kg. with a clamping period of 2 to 5 minutes is recommended for a relatively uncomplicated preparation.

This experiment was repeated many times with uniform results in protecting the bone marrow of the lower part of the body.† The results obtained on 5 representative rabbits are detailed below, and the blood counts are shown in Text-Figure 1.

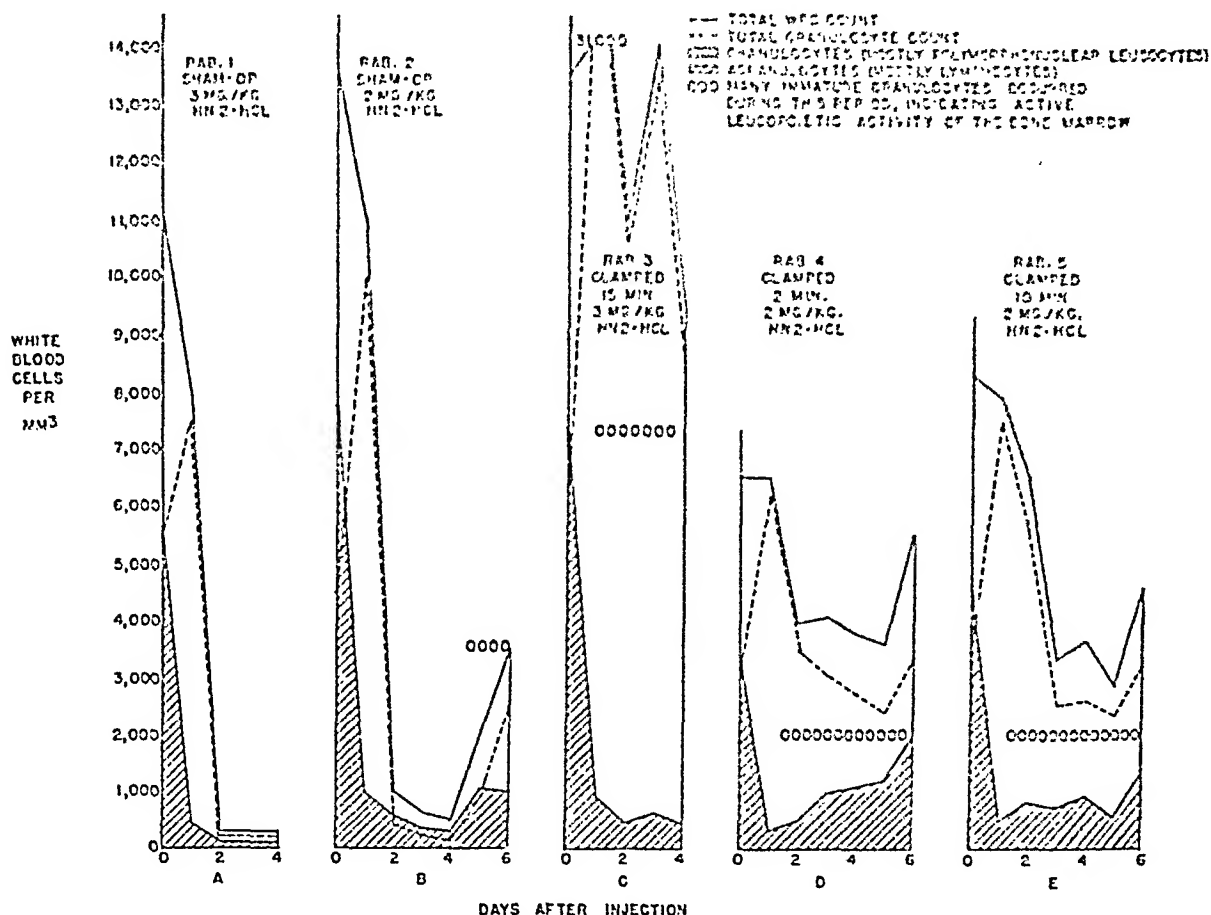
Rabbit 1. (A Sham-operated Control.) Rabbit 1 was given 3 mg. per kg. of $\text{HN}_2\cdot\text{HCl}$ intravenously. Diarrhea appeared on the second day, weakness was progressive, and death occurred 92 hours after injection. The daily leukocyte and differential counts (Text-Fig. 1-A)

* It is possible to produce occlusion of the abdominal aorta and vena cava by inflating a blood pressure cuff around the rabbit's abdomen. The use of this simplified technic has not been thoroughly investigated as yet.

† Preliminary experiments have shown that the reticulocyte count in the clamped rabbit injected with $\text{HN}_2\cdot\text{HCl}$ is severely depressed, although active regeneration of granulocytes is in progress. In view of the lymphocytotoxic action of the nitrogen mustards, this observation merits more detailed investigation.

were typical for the dose received. Typical pathologic findings were noted at autopsy, and there was no evidence of inflammation at the operative site.

Rabbit 2. (Clamp Applied on Vena Cava for 5 Minutes Only, 2 mg. per kg. Injected.) Rabbit 2 showed weight loss, diarrhea on the third day, and beginning recovery in weight 7 days after injection. The



Text-Figure 1. Effects of the direct action of nitrogen mustard on the bone marrow of the rabbit. A and B illustrate leukotoxic action of nitrogen mustard at two levels of dosage. In C, D, and E the protective effect of clamping the aorta and vena cava for varying periods during which similar dosages of nitrogen mustard were injected is illustrated.

leukocyte and differential counts are shown in Text-Figure 1-B and are in accord with our previous observations for this dosage. Nine days after injection the rabbit appeared almost completely recovered.

Rabbit 3. (Clamp Applied on Aorta and Vena Cava for 15 Minutes, 3 mg. per kg. Injected.) The day following injection, rabbit 3 showed generalized neurologic symptoms. The animal was weak and inco-ordinate in its movements, and these symptoms were present until death. Three days after injection a very severe diarrhea appeared, and the rabbit became progressively weaker, dying 104 hours after injection. The leukocyte counts were unprecedented, as shown in

Text-Figure 1-C. The lymphocyte count fell precipitously and recovery did not occur. Granulocytes were greatly increased in number, and within 24 hours there was an increase in banded cells, polymorphonuclear leukocytes containing basophilic granulations, macropolycytes, and occasional myelocytes, this picture persisting until death. Ninety-six hours after injection the leukocyte count was 9,000 and the smear showed occasional myelocytes and normoblasts, and the differential count showed 11 per cent banded and 83 per cent segmented polynuclear leukocytes, 3 per cent basophils, and 3 per cent lymphocytes. At autopsy no gross inflammation was found; the incision appeared to be healing, and the intestines, as was expected, were distended with fluid. Smears of the bone marrow showed an aplastic humeral marrow and a hyperplastic femoral marrow.

Rabbits 4 and 5. (Clamp Applied for 2 and 10 Minutes, Respectively, and 2 mg. per kg. Injected.) Rabbits 4 and 5 developed the usual picture of HN_2 intoxication, except for the fall in granulocytes. Banded polynuclear leukocytes and pseudo-eosinophilic granulocytes with basophilic granulations were present 24 hours after injection and persisted during the period of observation. One animal survived, and the other died with purulent peritonitis 12 days after injection. The total leukocyte and differential counts are shown in Text-Figure 1, D and E.

Comment

These studies demonstrate that the action of $\text{HN}_2\cdot\text{HCl}$ on the cells of the bone marrow is a rapidly completed one, probably accomplished within less than 2 minutes after injection. This strongly suggests, therefore, that this compound has a direct action on the hematopoietic cells. It further shows that systemic intoxication does not interfere with the process of active granulopoiesis. Also, the data make clear that leukopenia, *per se*, is not essential in the lethal effects of the tested compound.

The availability of a technic whereby the major portion of the hematopoietic tissue of the body may be temporarily destroyed, while preserving a small area of actively regenerating bone marrow, should be of considerable use in other hematologic investigations.

DEMONSTRATION OF THE DIRECT ACTION OF TRIS (β -CHLOROETHYL) AMINE HYDROCHLORIDE ($\text{HN}_3\cdot\text{HCl}$),* INJECTED INTRAVENOUSLY, ON THE INTESTINAL TRACT

Clamping experiments on the intestinal tract, similar to those described above with $\text{HN}_2\cdot\text{HCl}$, were carried out with $\text{HN}_3\cdot\text{HCl}$ in rats and rabbits. HN_3 produced effects similar to those reported for HN_2 ,

* $\text{HN}_3\cdot\text{HCl}$ is the official designation of the hydrochloride salt of this compound.

indicating that the former also has a rapidly completed action on the intestinal tract. It is probable that this will be true also in the case of the bone marrow.

DEMONSTRATION OF THE DIRECT ACTION OF BIS (β -CHLOROETHYL)
SULFIDE (H),* INJECTED INTRAVENOUSLY, ON THE INTESTINAL
TRACT AND BONE MARROW

H is poorly soluble and unstable in water. Water, therefore, was not a suitable vehicle for intravenous injection, and solvents, such as propylene glycol and thiodiglycol, were used. This factor seems to have complicated a simple interpretation of the results obtained by the clamping technic.

Methods and Results

Rats. It was first shown that by occluding the circulation to the small intestine during and for 5 minutes after the intravenous (inferior vena cava) injection of 2.5 mg. per kg. of neat H, the clamped portion of the gut was protected from injury. Similarly, clamping of the abdominal aorta for 5 minutes during and for 5 minutes after the intravenous injection of 1 mg. per kg. of H in propylene glycol protected the bone marrow distal to the clamp, whereas the bone marrow proximal to the clamp was destroyed. These results were in accord with those obtained with the nitrogen mustards.

Needham, Cohen, and Barrett⁹ reported, however, that they had been unable to obtain protection of the distal bone marrow in the rat against a dose of 2.0 mg. per kg. of H in thiodiglycol, injected intravenously, by applying a clamp to the abdominal aorta for 60 minutes. In order to resolve this discrepancy, 8 groups of 6 to 12 rats each were treated as shown in Table II. The results are summarized briefly therein.

This experiment confirmed both our previous experiment and the results obtained by Needham and co-workers.⁹ The protective effect of vascular occlusion was found only when a dose of 1 mg. per kg. of mustard in propylene glycol was given. With a higher dose of mustard, or with thiodiglycol as a solvent, the protective effect of temporary vascular occlusion was not demonstrated. The explanation of the failure to obtain consistent protection of the distal bone marrow in these experiments is not apparent.

Rabbits. Seven rabbits were subjected to the clamping procedure, and then injected intravenously with 4.0 mg. per kg. of H in propylene glycol (1.5 LD₅₀). In 2 rabbits the mesenteric arterial supply to a 15 cm. segment of the ileum was occluded during and for 15 minutes

*H is the official designation of this compound.

after the injection, and one rabbit was sacrificed at 72 hours and the other at 96 hours. These animals both showed extremely severe injury to the spleen, thymus, and bone marrow with terminal leukocyte counts of 900 and 350 per cmm., respectively. The portion of the intestinal tract subjected to the vascular occlusion was practically completely protected, whereas the remaining portion of the small intestine showed severe damage.

TABLE II

Protection Afforded the Femoral Bone Marrow of the Rat, by the Temporary Occlusion of Its Circulation, Against the Effects of the Intravenous Injection of H in Doses of 1 and 2 mg. per kg. in Propylene Glycol and Thiodiglycol

Group no.	No. of rats in group	Solvent used for injection	Intravenous dose of H	Duration of clamping	Effects on the bone marrow
A	6	None	mg./kg. None	minutes 5	None
B	6	Propylene glycol	None	5	None
C	12	Propylene glycol	1	5	Severe leukopenia, but the femoral marrow was less severely injured than the sternal
D	6	Propylene glycol	2	5	Femoral and sternal marrow seem to be equally severely affected
E	6	None	None	20	None
F	6	Thiodiglycol	None	20	None
G	6	Thiodiglycol	1	20	Femoral and sternal marrow seem to be equally severely affected
H	6	Thiodiglycol	2	20	Femoral and sternal marrow seem to be equally severely affected

Five rabbits had clamps applied to both the mesenteric artery and abdominal aorta during and for 15 minutes after the injection of H. One animal died soon after injection, and an autopsy was not performed in another dying 75 hours after treatment. Three of the 4 rabbits did not develop leukopenia, and at 72 and 96 hours, at the time of sacrifice, their leukocyte counts were 2250, 2850 and 4100 per cmm.; the remaining animal had a count of 600 per cmm. Histologic examination was performed on 3 rabbits. Again the temporarily clamped gut was protected, and the spleen and thymus in these animals were severely damaged. The rabbits all had extremely severe injury to the sternal, humeral, and upper vertebral bone marrow, whereas 2 rabbits showed complete and one rabbit (the one with a white count of 600 per cmm.) showed partial protection of the femoral bone marrow.

These experiments show, in general, what is much more clearly shown in the case of the nitrogen mustards, namely, that occluding the circulation to a given tissue before and for 15 minutes after the intravenous injection of mustard will serve to protect it from the action of the agent.

DEMONSTRATION THAT THE BACTERIOSTATIC EFFECT OF RABBIT SERUM
IS ALTERED BY THE INTRAVENOUS INJECTION OF HN₂*[†]

The demonstration that HN₂·HCl has a rapidly completed action and disappears from the blood shortly after injection makes it necessary to find a secondary mechanism which is more directly responsible for the development of systemic intoxication. A study of alterations in the circulating blood of intoxicated animals was undertaken, and the results have been reported elsewhere in this series.¹⁰ Among various approaches, the measurement of bacterial growth in serum as a possible method of demonstrating the appearance of toxic substances in the serum was considered. A simple experiment was performed, and this is briefly reported because of the unexpected results obtained.

Methods and Results

The growth of type A hemolytic streptococcus (C203) was determined in normal rabbit serum and in serum taken at various intervals after the intravenous injection of 3 mg. per kg. of HN₂·HCl. Blood was obtained by cardiac puncture, centrifuged, and the serum removed and refrigerated. One-tenth cc. of a 10⁻² dilution of an 18-hour culture of washed streptococci was implanted in 0.9 cc. of each sample of serum, and colony counts were made at 0, 4, 8, and 24 hours after incubation.

The control serum of 4 rabbits, whether starved or fed, was bacteriocidal, bacteriostatic, or permitted slight growth of bacteria over the period of 8 hours' incubation. In these same rabbits, 6 samples of serum drawn within 6 hours after injection of HN₂·HCl showed very little difference from the controls. Following this 6-hour period, and until the rabbits died, 3 to 5 days later, the serum became much more favorable for the growth of bacteria, and the rate of bacterial growth was strikingly accelerated.

Although the variability of bacterial growth in normal rabbit serum prevents any quantitative statement, enhanced bacterial growth of hemolytic streptococci occurred consistently in the serum of the intoxicated animals beginning 6 to 8 hours after the injection of an LD₅₀

* We wish to thank Dr. Colin MacLeod and Mrs. Edna Stone of the Department of Bacteriology, New York University College of Medicine, for assistance in carrying out this study.

dose of $\text{HN}_2\cdot\text{HCl}$. This observation suggests that some alteration occurs in the blood beginning at 6 to 8 hours after injection which facilitates the growth of the test bacteria. It was not possible to pursue this observation further.

SUMMARY AND CONCLUSIONS

1. Methyl-*bis* (β -chloroethyl) amine hydrochloride ($\text{HN}_2\cdot\text{HCl}$) was used as the type substance in this study. *Tris* (β -chloroethyl) amine hydrochloride ($\text{HN}_3\cdot\text{HCl}$) and *bis* (β -chloroethyl) sulfide (H) were examined in more limited trials and, in the particulars tested, gave essentially the same results as did $\text{HN}_2\cdot\text{HCl}$.

2. The "alarm reaction" of Selye must be considered in interpreting the toxic effects of any drug. It is shown that only a minor and questionable rôle can be ascribed to this reaction in the production of the characteristic pattern of injury to the lymphatic and hematopoietic tissues and intestinal mucosa resulting from $\text{HN}_2\cdot\text{HCl}$ intoxication. Following the injection of $\text{HN}_2\cdot\text{HCl}$ in the rat, involution of the lymphatic tissue and lymphocytic destruction occur in the absence of the adrenal glands.

3. That the damage to the intestinal tract and bone marrow is a rapidly completed and presumably direct action of the agent, and is not related to the "alarm reaction," was shown by the following procedures:

a. By means of occluding the circulation to a portion of the small intestine during and for 5 to 15 minutes after the injection of a lethal dose of $\text{HN}_2\cdot\text{HCl}$, that portion was protected from the damaging effect of the compound.

b. By means of occluding the circulation to the lower extremities by a clamp on the abdominal aorta and inferior vena cava during and for 2 to 15 minutes after the intravenous injection of $\text{HN}_2\cdot\text{HCl}$, granulocytopenia in the peripheral blood was prevented, and at autopsy hyperplasia of the femoral marrow, with aplasia of the rest of the bone marrow in the body, was observed.

4. Beginning 6 hours after the injection of a lethal dose of $\text{HN}_2\cdot\text{HCl}$ in the rabbit, the growth of hemolytic streptococci in the serum is enhanced. This suggests that an alteration occurs in the blood making it more favorable for the growth of bacteria.

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DESCRIPTION OF PLATE

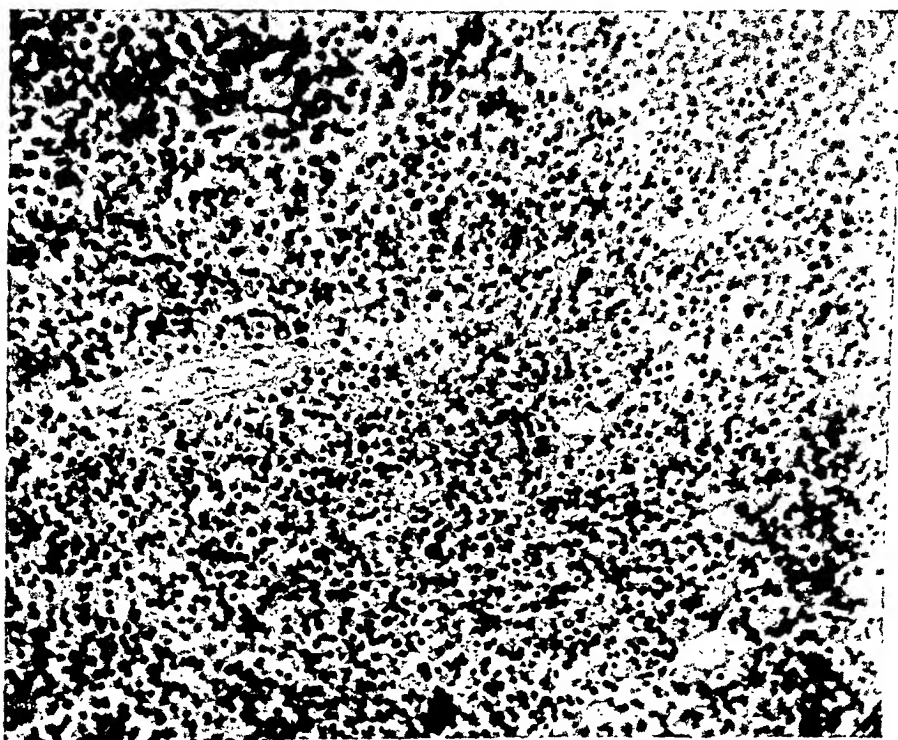
PLATE 57

- FIG. 1. Photomicrograph of a section of the sternum of a rat given 3.0 mg. per kg. of HN₂-HCl intravenously and sacrificed 70 hours afterward. The sinuoids are more prominent due to engorgement with red blood cells. The marrow is represented by fat spaces and cells and reduced numbers of stem cells imbedded in a matrix containing protein precipitate. Eosin and azure II stain.
- FIG. 2. Femoral bone marrow of a rat protected from intoxication by HN₂-HCl by clamping the aorta for 10 minutes during and after the intravenous administration of 2 mg. per kg. Eosin and azure II stain.

1



2



Karnofsky, Graef, and Smith

Effects of Nitrogen and Sulfur Mustards

HISTOLOGIC STUDIES ON A VIRILIZING TUMOR OF THE ADRENAL CORTEX *

EUGENE J. WEBER, M.D., AND MAUD L. MENTEN, M.D.

(From the Department of Pathology, University of Pittsburgh, and Children's Hospital, Pittsburgh, Pa.)

New growths of the adrenal cortex are frequently associated with an increased excretion of 17-ketosteroid hormones. Steroid hormones are formed normally in the cortical cells through a series of chemical transformations which parallel cytoplasmic changes, and are elaborated in excess by tumors. The neoplasms arise in different age periods, namely, fetal, juvenile, and adult. The fetal tumors arise in females and produce pseudohermaphroditism. The juvenile tumors have their origin in children of either sex and are associated with virilism and the precocious development of secondary male characteristics. Tumors of the adult give rise to heterosexual changes. The studies to be described deal with the histologic changes found in an adrenal cortical tumor, removed surgically, from a 4-year-old male, who showed advanced secondary sex characteristics and an increased urinary excretion of 17-ketosteroid hormones.

Reports of 21 authentic cases of virilizing adrenal cortical tumors occurring in males between the ages of 11 months and 16 years have been found in the literature.¹⁻²¹ Eight other cases have been recorded,²²⁻²⁵ but the data were insufficient to justify their inclusion among the proved cases. The tumor was removed surgically from 9 patients^{4, 5, 12, 18, 21} with 2 survivals,^{16, 18} and hormone assays were reported from 5 patients.^{3, 5, 12, 18, 21} Seventeen of the records included brief histologic descriptions except in the report of Goormaghtigh.⁶ In a study of the cellular characteristics of adrenal cortical neoplasms, Goormaghtigh described in 2, virilizing tumor cells showing transformation of mitochondria into granules which gradually were converted into clear droplet-like structures. These droplets, after the discharge of their contents into the neighboring lymph channels, remained as vacuoles. He had previously published a study on the chronologic cellular development of the adrenal cortex and later attempted to correlate the cytologic features of the normal tissue with those found in different adrenal cortical tumors.²⁶

CLINICAL HISTORY

P. M. P., a white male, 3½ years of age, was admitted to Children's Hospital on March 9, 1945, as a patient of Dr. J. W. Leech. The patient had had a gradually enlarging abdomen since birth and a generalized growth of fine hair, but the

* Received for publication, April 30, 1947.

latter had not attracted particular attention. He was the first of two children born to apparently normal parents, and no similar conditions were known in any member of either family. The past medical history was not pertinent. Physical examination revealed a well developed musculature. In the right upper quadrant of the abdomen, a large mass was felt which extended 5 cm. below the costal margin in the mid-clavicular line. This mass showed, roentgenographically, some fine areas of calcification and was tentatively diagnosed as liver. Other physical findings, except a moderate tachycardia, were normal. After 1 week, the patient was discharged to be observed at home. In May the parents consulted Dr. Joseph Stokes, Jr., of Philadelphia who, on the basis of pycnographic examinations, diagnosed the mass as a tumor of the right adrenal.

The patient was readmitted on October 2, 1945. At that time a definite increase in the growth of the beard and the pubic and axillary hair was noted. The genitalia resembled those of a 16-year-old male. Acne had developed over the face and neck. The blood pressure was 190/160 mm. Hg. The right ocular fundus was swollen and hemorrhagic with an obscured optic disk. The urine and blood cellular pictures were normal. The bone age was approximately 4 years. On October 5, an exploratory laparotomy was performed by Dr. J. W. Shirer. The liver was displaced downward and forward by a large, nodular, encapsulated mass in the region of the right adrenal. This mass was separate from the kidney and was not attached to the diaphragm. No evidence of metastasis was seen in the liver, and the left adrenal appeared normal in size and shape. On October 5, a steroid assay by a modified Zimmermann²⁷ method on a 24-hour urine specimen gave a value of 14.7 mg. of 17-ketosteroid. On October 15, the tumor was removed by Dr. Shirer through a flank incision, and the patient made a rapid recovery with no evidence of adrenal cortical insufficiency. After operation, the blood pressure fell to 90/60 mm. Hg and has remained in this range. On October 17, a 24-hour urine specimen gave 5.4 mg. of sodium pregnandiol glucuronidate on an analysis by the Venning method.²⁸ There was no precipitable free steroid. The patient was discharged from the hospital on October 28, 1945.

On November 14, 1945, the 17-ketosteroid level was 4.8 mg. with a 24-hour output of 600 cc. of urine, and on January 2, 1946, a similar low level of 4.7 mg. was obtained on a 24-hour output of 274 cc. of urine.* The normal urinary 17-ketosteroid level for a 3½-year-old male is 5 mg. for 24 hours and for an adult male, 30 mg. per 24 hours.^{29,30}

Pathologic Data

The tumor, which included the right adrenal, was an encapsulated, nodular mass, measuring 10 by 10 by 7 cm. The blood supply entered through a pedicle. The cut surface showed two types of tissue: one, which comprised most of the tumor, appeared yellow and fatty with many small stellate areas of calcification; and a second, which consisted of small peripherally located areas, was reddish and relatively soft and friable. The tumor was preserved in Jores' solutions, and blocks were fixed in Zenker's solution, and in equal parts of 95 per cent alcohol and 10 per cent formalin. The stains used on fixed tissue were hematoxylin and eosin, Masson's trichrome, ponceau fuchsin and aniline blue, iron hematoxylin, and van Gieson's. Frozen sections were stained with sudan IV, osmic acid, and ponceau fuchsin.

* We are indebted to Dr. H. S. Strickler, Department of Biochemistry, University of Pittsburgh School of Medicine, for these analyses.

Microscopically, the tumor was made up of an anastomosing network of tissue enclosing vascular channels, architecturally similar to the zona reticularis of the normal adrenal cortex. The strands of the network were composed of two main types of tissue; one predominantly cellular (Fig. 1), and the other hyalin-like and relatively acellular (Fig. 2). Various intermediary stages were found between these two types (Fig. 3). The cellular portion ranged from thick double-celled strands of polygonal cells (Fig. 1) through gradually narrowing cords (Figs. 3 and 4) to small, shrunken, degenerated cells with pyknotic nuclei (Figs. 2 and 3). The degenerated forms were the end result of progressive secretory changes. In the normal gland, the secretory phases, which have been designated pre-secretory, secretory, post-secretory, and senescent, occur chronologically and are aligned with definite cytologic characteristics. In the tumor studied, comparable cytologic patterns, with the exception of the earliest cell type, have been established. Eight different cell types were demonstrated. Six of the eight types occurred in the cellular portion, and the remaining two were senescent cells associated with the hyaline cords. The cells and nuclei of the cellular tissue varied in size depending upon the phase of activity. The largest cells occurred in the secretory phase. The earliest stages of the pre-secretory phase were difficult to find, and the parent cells, which originate in the capsule of the normal adrenal, could not be identified with certainty. What we believe to be the immediate successors of the parent cell were found, but in small numbers only. This earliest identified cell type belonged in the pre-secretory phase and averaged $10\ \mu$ in diameter. The nuclei, which were occasionally paired, were relatively large, and the cytoplasm was compact, homogeneous, and stained diffusely with eosin (Fig. 6a). The next development was the formation throughout the cytoplasm of dust-like indistinct granules which gave the cytoplasm a *finely granular*, pink appearance when stained with ponceau fuchsin (Fig. 6b). These granules form the initial stage in the genesis of the mitochondria. This pre-mitochondrial material gradually became segregated as larger, irregular granules staining black with iron hematoxylin and red with ponceau fuchsin (Fig. 6c). These granules, which apparently arose through cohesion of the denser components of the cytoplasm, gradually became more compact and were surrounded by a clear spherical zone (Fig. 6d). The four successive cell changes ending with the establishment of the discrete granules constituted the pre-secretory phase.

Gradually each distinct, small mitochondrial mass was transformed into a large globule, staining red with sudan IV or ponceau fuchsin. This globular formation constituted the secretory phase. The cells of

the secretory phase reached approximately $25\ \mu$ in diameter, and this increase in size apparently was due to expanding secretory globules. The largest globules were found adjacent to the cell wall bordering the vascular channel, and the smaller ones deeper in the cytoplasm (Fig. 7a, b, c). The globules were bounded by a more deeply staining, thin peripheral layer. When observed with polarized light, some of the globules contained, or were intimately associated with, doubly refractile material, obviously cholesterol. In the frozen sections, the globules stained magenta red with ponceau fuchsin and orange with sudan IV. With routine stains, the globules were not discernible, although in the denser areas of the cytoplasm diffuse, reddish-staining, finely granular material was observed. The contents of the globules were readily soluble in fat solvents and were not retained within the cell after prolonged fixation (Fig. 5) or after treatment with xylol. When xylol was applied to frozen sections stained with ponceau fuchsin, the outward diffusion of the reddish contents could be seen readily. In some of the frozen sections made from tissue fixed for a relatively short time and stained with ponceau fuchsin, masses of homogeneous red material, which had either been secreted *in vivo* or had diffused from the contiguously lying cells during fixation, were seen in the vascular channels. The latter process seemed to be the more probable. Frozen sections made at successive intervals during prolonged fixation showed a diminishing capacity of the globular contents to stain. The staining capacity was completely lost in tissue after fixation for 4 months and the secretory cells contained mainly empty vacuoles (Fig. 5). With the *in vivo* diffusion of the contents of the secretory globule into the blood stream, the secretory phase was ended. The post-secretory cell was smaller than that of the previous phase, and cells could sometimes be found in which the cytoplasm contained empty vacuoles with definite boundaries (Fig. 8).

The senescent phase was characterized either by swelling or shrinking. When swelling occurred, the nuclei became enlarged and karyolytic, following which the cells appeared fragmented and disappeared (Fig. 9a). When the nuclei became pyknotic, there was a diminished cytoplasm in which fibrils occasionally appeared (Fig. 9b). These fibrils stained blue with Masson's stain or red with eosin and apparently arose from the dark-staining walls of the post-secretory vacuoles. These fibrils varied in number and thickness, and were associated with the developing hyalin. In some of the degenerating cells stained by Masson's method, transformation of the cytoplasm into hyalin could be followed by variations in color (Fig. 10). The normal reddish-colored cytoplasm became bluish in patches in which the fibrils were embedded.

These blue patches extended and finally filled the entire cell. With the conversion of the cytoplasm into hyalin, remnants of the finally depleted senescent cells were seen either as a persistent thin network of pyknotic nuclei with a minimal amount of cytoplasm bordering the vascular channel, or as a conglomeration of cast-off nuclei lying within the vessel. These desquamated cells were very evident in sections stained with iron hematoxylin. It was difficult to establish whether the fibrils arose from the walls of the empty vacuoles or whether the degenerating cytoplasm contributed to their formation. The cellular changes following secretion were rapid and difficult to follow with certainty. The amount of hyalin was in inverse proportion to the quantity of residual cytoplasm in the degenerating cellular elements.

DISCUSSION

In the adrenal cortex, an orderly progression of cells from the capsular region through the glomerular and fascicular zones to the zona reticularis has been recognized since 1883.³¹ Studies concerned with this progression were reported by Hoerr,³² Bachmann,³³ and Wotton and Zwemer,³⁴ all of whom indicated that the primary cell migrated through the cortex and during its migration underwent metabolic and morphologic changes with final eradication. Goormaghtigh,⁶ Bennett,³⁵ and Swinyard³⁶ have added the minute cytoplasmic changes which occur in chronologic order throughout the progression. The cytoplasmic transformations are associated with the chemical formation of a progressive series of steroid hormones. There is little definite information available at present concerning this series, the development of which is under investigation by various workers. The cellular changes, which have been followed in the tumor studied, roughly duplicate those in the normal cortex; however, changes in the normal cortex occur in an orderly progression, divisible into anatomic zones, but those in the tumor, although they are represented by similar cell types, do not occur in an orderly architectural fashion.

Our most interesting observations deal with the fatty secretory globules, which stain with ponceau fuchsin and are identical with those described by Broster and Vines^{3,37} with this stain. These authors believed that the positive staining reaction was a definite indication of the presence of a male steroid hormone, a postulate which has not been substantiated by other workers to date. The material contained in the globules is readily soluble in fat solvents and is dissolved from the cell in any technic requiring paraffin embedding. The only procedures which gave positive globular staining in our hands were those carried out on frozen sections. Such material staining with ponceau

fuchsin is not only readily soluble in xylol but also soluble, although in less degree, in aqueous solution. It is interesting that frozen sections, made from the tumor material shortly after placing in Jores' solution II, contained globules filled with deeply-staining material. After some weeks of immersion in Jores' solution there was a marked reduction in stainable material in the cells. The rapid outward diffusion of the stained contents of the globules when placed in suitable solvents demonstrated the lability of the contents and explained the formation of the vacuoles. The presence of vacuoles with a lack of stainable content in the carcinoma of the adrenal cortex studied by Goormaghtigh²⁶ is probably explained by the diffusion of the globular content during preservation and fixation. Attempts by us at histochemical demonstration of androsterone *in situ* were not successful because, first, the amounts in the tissue were decreased by diffusion, and, second, the reagents used drastically altered the tissue. Proof of the elaboration of male sex hormones by the tumor is indicated by the functional and anatomic changes induced by the tumor and their disappearance on removal of the new growth.

The recent work of Mason and Kepler³⁸ demonstrates that in a majority of the virilizing tumors of the adrenal cortex, the predominating urinary hormone is dehydro-iso-androsterone with a small amount of androsterone. The presence of dehydro-iso-androsterone in large amounts in urine is practically diagnostic of virilizing adrenal cortical tumors. The association of cholesterol with the material staining with ponceau fuchsin suggests cholesterol as a basic substance, which by oxidation gives rise in the adrenal cortex to a series of adrenal steroids numbering at least 30.³⁹

SUMMARY

In a virilizing adrenal cortical tumor removed from a boy, 3½ years old, a progressive series of cells were identified which paralleled in morphologic sequence and associated secretory changes a comparable series of cells occurring in the normal gland. The secretion, which was associated with the formation of hormones, could be demonstrated histologically with ponceau fuchsin and sudan IV and was removed from the cell by fat solvents.

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DESCRIPTION OF PLATES

PLATE 58

FIG. 1. Islands of secretory cells surrounded by endothelium-lined sinuses which contain red blood cells. $\times 700$.

FIG. 2. Hyaline cords containing remnants of a few degenerating nuclei and many extruded senescent cells in different stages of degeneration bordering the sinuses. $\times 700$.

FIG. 3. Gradual degradation of secretory cells into hyalin. Cells in the secretory phase are shown in the upper third of the section. In the central part the cells are in the process of post-secretory degeneration. The cells in the lower third are undergoing hyalinization. $\times 225$.

FIG. 4. Early stage in the degeneration of secretory cells. $\times 700$.

FIG. 5. Vacuoles in tissue from which the secretory contents have diffused during a fixation period of 4 months. $\times 225$.

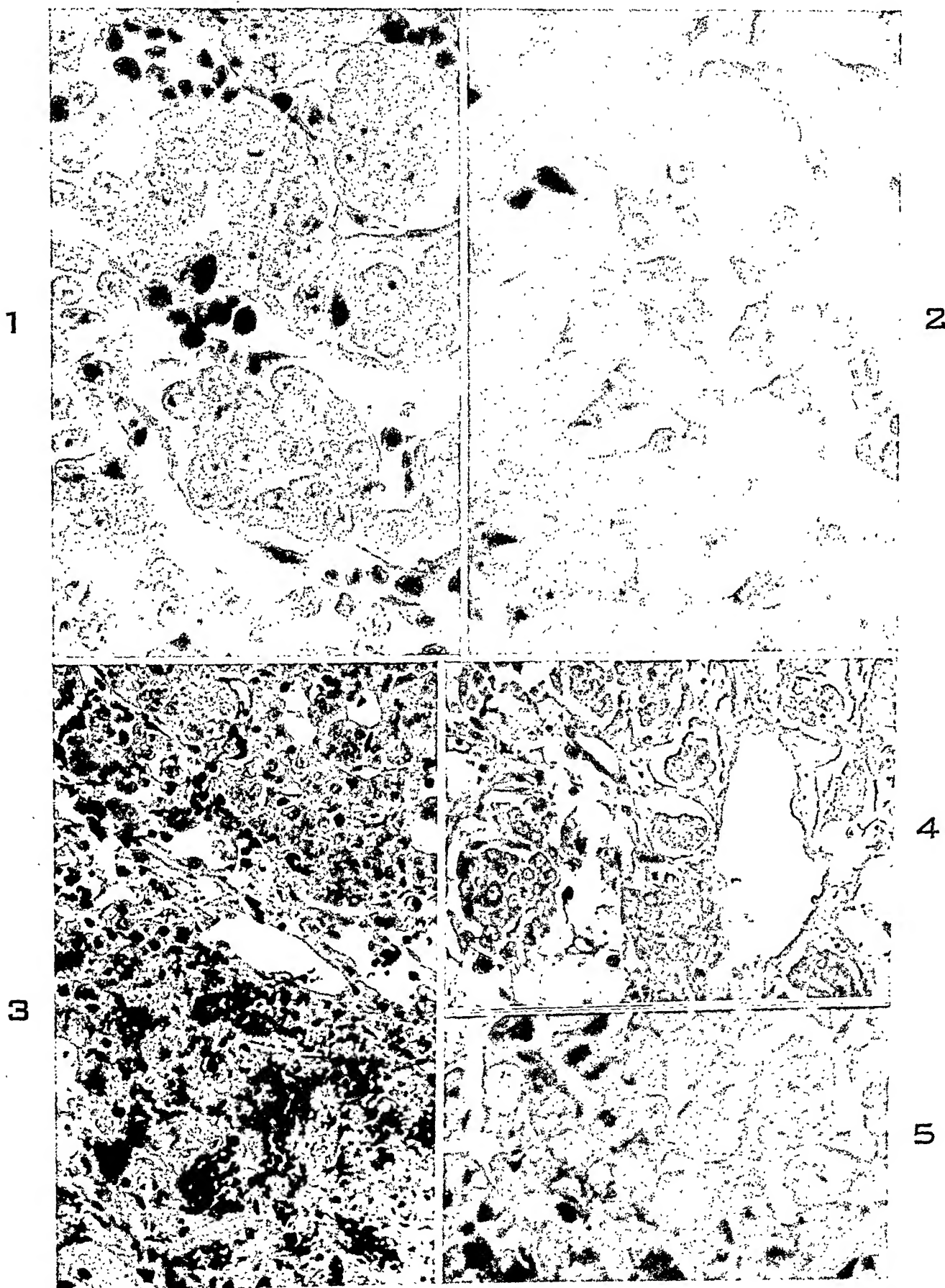


PLATE 59

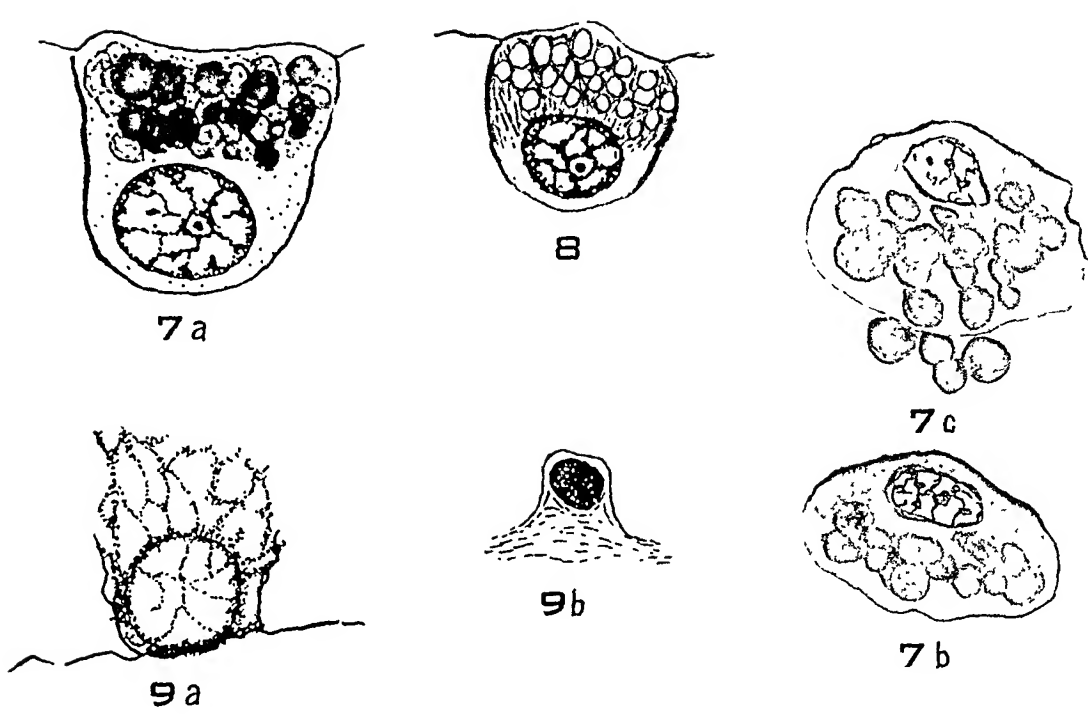
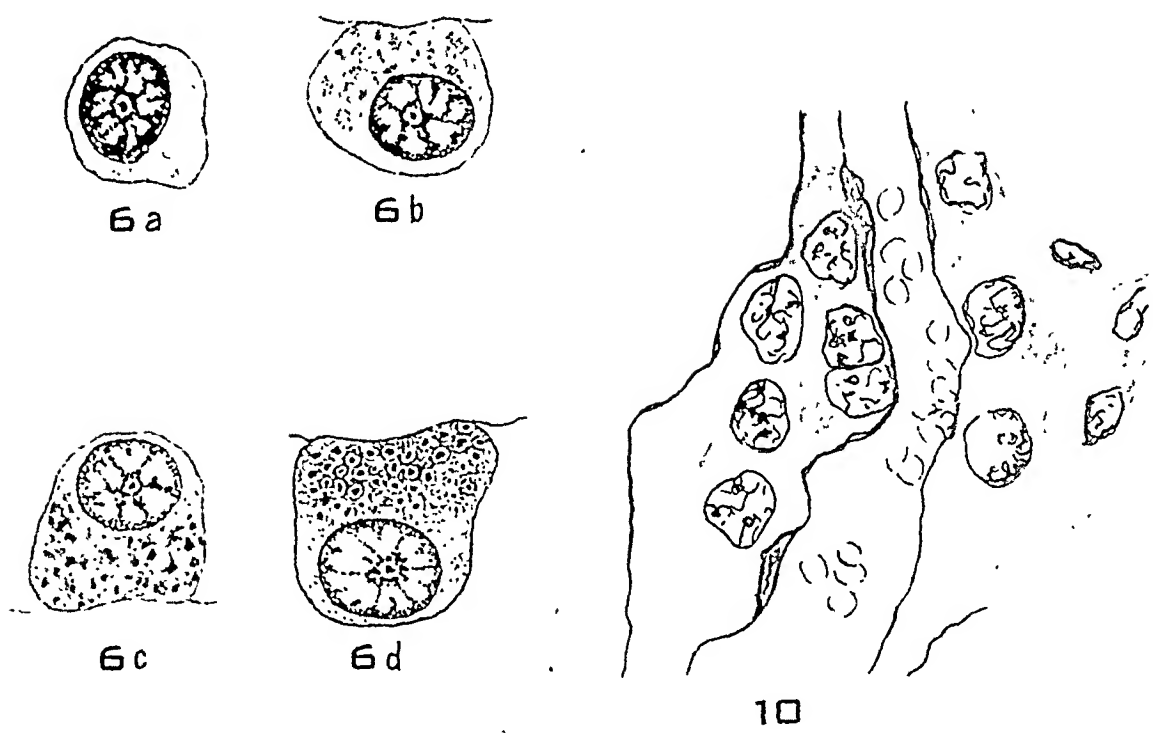
FIG. 6a, b, c, d. Photographs of camera lucida drawings showing the progressive development of granules in the pre-secretory phase. $\times 1800$.

FIG. 7a, b, c. Photographs of camera lucida drawings of cells in the secretory phase showing (a) secretory globules in cytoplasm bordering vascular channel, (b) globules immediately before expulsion of contents, (c) extrusion of globular contents into sinus. Globules stain magenta red with ponceau fuchsin stain. $\times 1800$.

FIG. 8. Photograph of camera lucida drawing showing vacuoles in a post-secretory cell after contents have been secreted into a blood sinus. $\times 1800$.

FIG. 9a, b. Photograph of camera lucida drawing of senescent cells showing (a) fragmentation of cytoplasm and karyolysis of nucleus, (b) shrinking of cytoplasm and pyknosis of nucleus. $\times 1800$.

FIG. 10. Photograph of camera lucida drawing showing the formation of hyalin in the cytoplasm of two senescent cells bordering a sinus. The homogeneous cytoplasm (stained red in section) is being converted into fibrils (stained blue in section). Masson's stain. $\times 1800$.



Weber and Menten

Virilizing Tumor of Adrenal Cortex

TUMORS OF THE CAROTID BODY *

PHILIP M. Lecompte, M.D.†

(From the Laboratories of the New England Deaconess Hospital and the Harvard Cancer Commission, Boston, Mass.)

Tumors arising in the carotid body or glomus caroticum have been called by many names, including perithelioma, endothelioma, angioma, chromaffinoma, adenoma, paraganglioma, pheochromocytoma, and sympathoblastoma. The variety of names reflects the current confusion regarding the development, structure, and function of the carotid body. Accordingly, a consideration of some of the contemporary ideas on these subjects is in order.

Embryology

The carotid body was regarded by Kohn²⁵⁻²⁷ as being derived from cells of the sympathetic nervous system and from the same anlage as the sympathetic ganglia. This concept has dominated the field until recent times, when considerable doubt has been cast upon it.

Smith⁴² regarded the carotid body as a complex of elements which become associated during the developmental history of the third mesodermal arch. She indicated that mesodermal cells and neural elements from the glossopharyngeal, vagus, and sympathetic nerves might all participate. Boyd⁴ emphasized a mesodermal condensation related to the third branchial arch artery and indicated his belief that these cells persist to form a considerable portion of the essential cells of the adult structure. He acknowledged also contributions from glossopharyngeal, vagus, and sympathetic nerves, but felt that these were of secondary importance.

The possibility that a few sympathetic cells are included in these bodies cannot be denied, especially since, as noted by Hollinshead,¹⁹ the difficulties of following wandering embryonic cells in the region of the branchial arches are considerable. It seems probable, however, that even if migratory elements from the sympathetic ganglia are included in the carotid body, their rôle is a minor one.^{4, 19, 42}

Anatomy

Grossly, the carotid body is a soft, but tough, ovoid, pale tan, rather poorly defined mass measuring about 5 by 3 by 2 mm. It is situated usually a little medial to and behind the bifurcation of the common

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† Now at Faulkner Hospital, Jamaica Plain 30, Boston, Mass.

carotid artery, being rather firmly bound to the vessels by loose fibrous tissue.

Microscopically, the tissue may be arranged in more or less distinct lobules (*e.g.*, cow, man) or in a compact mass (cat). The essential or "specific" cells are arranged in whorls or clusters (called "Zellballen" by Kohn²⁶) which are completely surrounded by a supporting stroma exceedingly rich in capillaries (Figs. 1 and 2). This fundamental and characteristic structure is brought out clearly by silver impregnation of the reticulum (Fig. 3). The cells making up these clusters are round or polyhedral, resembling epithelium, with round or slightly oval nuclei containing fine chromatin particles. Their cytoplasm is abundant and may appear granular, vacuolated or reticular, depending in part upon fixation and technic. Cell boundaries are usually distinct, and there appears to be no good evidence that they ever form a syncytium. The presence of ganglion cells^{15,33} has been seriously questioned by Hollinshead.²¹

The supporting stroma consists of fine collagenous and reticular fibers with innumerable capillaries. The endothelial cells of the latter are so large and numerous that some may appear to lie outside of the capillary lining (the so-called "perithelial" cells). Some of them are perhaps homologous with the "neuro-myo-arterial" cells of the cutaneous glomus.

In specially prepared sections, numerous nerve fibers are found. Thus, the principal constituent elements of the carotid body may be considered to be the "chief" or epithelioid cells making up the cell clusters, and the neural and endothelial elements (the latter perhaps including "perithelial" cells).

The Chromaffin Reaction

Much of the confusion regarding the nature of the carotid body and the histogenesis of its tumors arises from the use of the so-called chromaffin reaction. This refers to a yellowish brown color assumed by certain cells after fixation in fluids containing chromium salts. The prototype of this reaction is seen in the cells of the adrenal medulla, where the material taking the brown color is regarded as a precursor of epinephrine. Other cells giving the reaction are found in association with sympathetic ganglia, particularly in the abdomen.

Kohn²⁵ laid particular emphasis on the presence of the chromaffin reaction in some cells of the carotid body in various animals and regarded this as important evidence for his assumption of a sympathetic origin for the specific cells. Cells not giving the chromaffin reaction were regarded as of sympathetic origin nonetheless. Smith⁴² and Boyd⁴ found the chromaffin reaction to be present in a portion of the

cells of the carotid body in certain species (*e.g.*, cow, pig), but absent in others (*e.g.*, rat, man). DeCastro,^{7,8} in a careful study, questioned the existence of a true chromaffin reaction in the carotid body and stated that the apparent reaction was due to the presence of lipid substances in the cells. This conclusion was largely confirmed by Hollinshead,²¹ who, however, felt that the cytoplasmic granules were not lipid in nature, but might be mitochondria.

The chromaffin reaction has been clearly shown to be nonspecific. Indeed, the word "chromaffin" is a misnomer, since various oxidizing agents other than chromium salts will give the reaction.¹² Furthermore, organic substances other than epinephrine will form brown compounds when oxidized, *e.g.*, hydroquinone, resorcinol, aniline, various aldehydes and ketones, polyphenols, and polyamines.¹

Innervation

The innervation of the adrenal medulla, with which Kohn²⁵ supposed the carotid body to be homologous, is motor and predominantly preganglionic. The carotid body, on the other hand, receives chiefly sensory nerves from the glossopharyngeal nerve.⁷

Function

The carotid body is sometimes referred to as a gland of internal secretion¹¹ or as having no known function.⁴³ The suggestion of DeCastro⁸ that it is a chemoreceptor apparently has received abundant confirmation in the work of Heymans and Bouckaert,¹⁸ of Schmidt and Comroe,⁴¹ and of Dripps and Comroe,⁹ who have shown that it and the similar cardio-aortic bodies are sensitive to changes in the pH, and in the carbon dioxide and oxygen tensions of the circulating blood, and that under certain conditions they may be of major importance in the regulation of respiration. It is also noteworthy that extracts of the carotid body have not been shown to contain epinephrine.⁵

Classification of the Carotid Body

Kohn's classification of the carotid body with the "paraganglia"²⁵⁻²⁷ has had great influence down to the present day. Apparently he used the term to apply to collections of tissue associated developmentally with the sympathetic ganglia and homologous with the adrenal medulla. As noted by Hollinshead,¹⁹ if this classification is to be acceptable for the carotid body, the latter should be shown to be similar to the adrenal medulla in development, structure, innervation, and function. However, as outlined above, the two structures appear to differ on all these counts. Similar observations apply to the aortic bodies or cardio-aortic bodies,^{3,36,37} and it seems extremely doubtful that either they or the

carotid body should be included with the paraganglia. The propriety of regarding the carotid body as an arteriovenous anastomosis¹⁵ or "glomus" is also questionable, since Hollinshead²⁰ has shown that it differs in vascular arrangement, innervation, and cytologic features from the glomus coccygeum, which presumably does represent a specialized arteriovenous anastomosis. It appears, therefore, that the carotid body, together with the similar cardio-aortic bodies, should be classified as a specialized chemoreceptor and not as a gland of internal secretion, paraganglion, or arteriovenous glomus.

TUMORS

The present report is based upon 17 tumors taken from the files of the New England Deaconess Hospital and the Laboratory of Pathology of the Harvard Cancer Commission.* Without exception, they were removed surgically from the region of the bifurcation of the common carotid artery. In one case, only tissue taken for biopsy was available.

Gross Appearance

Weights were not recorded for most of the tumors. Three weighed 28, 50, and 60 gm., respectively. Measurements varied from 3 by 3 by 2 to 5 by 4 by 3 cm. In most cases the shape was globular or ovoid, a groove for the carotid artery sometimes being present if the artery had not actually been resected. In cases in which it was necessary to remove a segment of the artery, the neoplastic tissue was found to be closely adherent to the wall of the vessel. A well defined capsule usually was present. The color was recorded as pinkish gray to reddish brown. The tissue was usually firm and homogeneous.

Microscopic Features

Microscopically, most of the tumors showed a tendency to mimic the structure of the normal carotid body. The usual pattern was one of nests of the "chief" or epithelioid cells, fairly uniform in size, and surrounded by a vascular stroma similar to that of the normal organ. There was usually more variation in size and shape of the cells than in the normal gland. Mitotic figures were not observed. Generally speaking, the cell clusters of the tumors were larger than those of the normal organ (Figs. 4 and 5), and the supporting stroma was less cellular. The general pattern is best brought out by silver impregnation of the reticulum (Figs. 6 and 9).

For descriptive purposes, it was found possible to subdivide the tumors into three groups. The first, or *usual* type, included most of

* The clinical aspects of 15 of these cases have been reported separately by Lahay and Warren.²⁰

the cases (12 tumors) and is the variety in which there is more or less faithful reproduction of the normal structure (Figs. 4, 5, 7, and 8). The second, or *adenoma-like* type (2 tumors), shows a pattern in which the chief cells have a pronounced epithelial appearance, with rounded or polyhedral shape, abundant cytoplasm, and arrangement in sheets or rows (Figs. 10 and 11). This appears to be the type referred to as adenoma by some authors.¹¹ In this type the reticulum is scanty. The third type may be called *angioma-like* (2 tumors). Here the cells are largely spindle-shaped or crescentic and apparently closely related to capillaries (Figs. 12 and 13).

In spite of these variations, however, the fundamental pattern appeared to be the same. The structural unit consisted of a group, of variable size, of the chief or epithelioid cells, surrounded by a more or less abundant vascular stroma. Most of the variations in microscopic appearance of the tumors seemed to be produced by differences in the shape of the chief cells. This in turn was apparently influenced to no small degree by such factors as clamping and squeezing the tissue during surgical removal, the time elapsing between removal and fixation, and the type of fixative. In many instances, more or less flattened or spindle-shaped cells were found in part of a tumor, while in better preserved fields the cells were plump and rounded. The mechanical factors involved in the growth of a well encapsulated tumor in an environment naturally restricted by dense fascial planes may also be considered as influencing the shape of the cells.

Most striking was the persistence of the general pattern of the reticulum in the majority of cases (Figs. 6, 9, and 13). Although the number of fibers and the size of the cell groups which they surrounded varied, the basic arrangement remained the same.

The Chromaffin Reaction

The cytoplasm of the chief cells was, in varying degrees, granular, vacuolated, or reticulated. Stains for fat and glycogen in several instances were negative. Vacuolation was often most marked in poorly fixed specimens. In many cases attempts were made to bring out a "chromaffin" reaction in the cells. In those cases in which fresh tissue was available, the technics described by Bennett¹ were used, such as fixation in formol-dichromate solution followed by mordanting for several days in 3 per cent potassium dichromate, and in some cases by "intensification" in Fontana's ammoniacal silver solution. When only fixed tissue was available, paraffin sections cut from formalin-fixed blocks were mordanted on the slide in 3 per cent solution of potassium dichromate, as recommended by Lison.³²

In almost all instances, tissue treated with dichromate solution had

a faint yellowish color when compared with untreated tissue. However, this was never as striking as the color developed by the adrenal medulla under similar circumstances. Also the color of the neoplastic tissue was generally not more intense than that shown by sections of various other organs (*e.g.*, liver, heart, kidney) treated in the same way. No convincing evidence of a true chromafin reaction could be found. The nonspecificity of the reaction has been commented upon above.

Assay for Epinephrine

Assays* for epinephrine were carried out on 2 of the tumors. In each case the fresh tissue was frozen solid within 2 or 3 hours after surgical removal and kept in that state until the assay was performed. This was done by extracting the tissue with 0.01 N hydrochloric acid and comparing the effect of this extract with a known solution of epinephrine. Tests were done on the blood pressure of the spinal cat and on the isolated strip of ileum of the rabbit. The first tumor (no. 76947) showed an activity equivalent to less than 8 γ of epinephrine per gm. of tissue, *i.e.*, a negligible amount. The second (no. 87076) was found to have an epinephrine-like action corresponding to about 0.4 mg. per gm. on the basis of blood pressure tests and 0.25 mg. on the basis of the test with the strip of ileum. The cause of the discrepancy between the two tumors was not apparent. Further tests on the second tumor, however, indicated that the pressor substance in question was probably not epinephrine.

Nerve Endings

An abundance of nerve endings has been demonstrated in the normal carotid body.^{7,8} Apparently, nerve endings are absent in the tumors, although few attempts have been made to demonstrate them.³ In one case in the series here reported, fresh tissue was fixed in 25 per cent chloral hydrate and impregnated according to one of the methods of DeCastro.[†] In one of several sections a few ill defined club-shaped structures were seen, but no definitely recognizable nerve fibers or nerve endings.

Evidence of Malignancy

None of the tumors showed evidence of malignancy. As noted above, practically all of the tumors were described as well encapsulated and as having a fairly smooth surface. Although many of the tumors were intimately adherent to the carotid arteries, no definite invasion of sur-

* I am grateful to Drs. Otto Kraye, Ralph Brauer, and Harriet M. Maling of the Department of Pharmacology of the Harvard Medical School for these assays.

† I am indebted to Dr. James Goddard for advice concerning this technic.

rounding structures was recorded in any case. In 5 cases, lymph nodes, varying in number from 1 to 6, were removed with the tumor mass. None showed microscopic evidence of invasion by neoplastic cells. No evidence of distant metastasis was present in any case.

Mitotic figures were not seen in any of the tumors. In 8 of 17 cases there was fairly marked variation in nuclear size (Fig. 14), with occasional giant forms.

DISCUSSION

The ideas of Kohn²⁵⁻²⁷ on the origin, structure, and function of the carotid body have dominated almost all discussion of the organ down to the present day. The important work of Hollinshead,¹⁹⁻²² outlined briefly above, has been overlooked by even the more recent writers on carotid body tumors, with the notable exception of Bloom.³ The physiologic evidence provided by Heymans and Bouckaert¹⁸ and Schmidt and Comroe⁴¹ has likewise not been emphasized. It seems clear from the work of these investigators that the carotid body is a chemoreceptor, not a gland of internal secretion; that its embryologic origin, while not entirely established, is probably not primarily from sympathetic elements; that it probably does not give a true chromaffin reaction, at least in man; that its innervation is primarily sensory; and that it does not secrete epinephrine.

There would appear, likewise, to be no satisfactory evidence that the tumors of the carotid body are derived from tissue of sympathetic origin. They resemble only superficially the pheochromocytomas of the adrenal medulla, and do not resemble at all the neuroblastomas of sympathetic origin.* Efforts to demonstrate the chromaffin reaction have been scanty and unconvincing. Most writers have been limited to formalin-fixed tissue and have regarded it as unsuitable for bringing out the chromaffin reaction, although in fact it is not.³² Painstaking attempts like those of Bloom³ (who failed to demonstrate the reaction) have been the exception rather than the rule. Likewise, efforts to demonstrate epinephrine by bio-assay or by chemical methods have met uniformly with failure, although a substance having a depressor effect on the blood pressure has been found⁵ and in one of the tumors here reported a pressor substance of unknown nature was encountered (see above).

As for *nomenclature*, it would appear, in the light of available evidence, that terms such as "chromaffinoma,"^{14,34} "paraganglioma,"¹¹⁶ and "adenoma"¹¹ are of doubtful propriety. The question whether

* The report by Cragg⁶ of a carotid body tumor occurring simultaneously with tumors of the organs of Zuckerkandl is of great interest but can hardly be regarded as providing much evidence regarding the nature of carotid body tumors.

these tumors are to be regarded as hamartomas is raised by Kaufmann and Ruppner,²³ and dismissed by the same authors because of the failure of investigators to demonstrate nerve endings in the tumors. The evidence in favor of the term "perithelioma" is discussed by Ewing.¹⁰ However, the desirability of referring to the constituent cells as occurring "around blood vessels" when actually it is the blood vessels which surround nests of the "chief" cells, is open to question. As noted above, these cells probably may assume a spindle-like or flattened form resembling endothelium because of mechanical factors. Certainly they may, and do, take on a globular epithelium-like appearance in many instances. Also, the embryologic evidence is not in favor of an origin of these cells from endothelium or from adventitial cells in the walls of blood vessels.⁴ The stroma of these tumors is admittedly exceedingly vascular, but, even in the "angiomatous" type described above, there seems to be no good reason for regarding it as essentially neoplastic or as differing in any important qualitative way from the stroma of other tumors. On the whole, it would appear that, unless the "chief" cells of the carotid body can be shown to be of the same nature as the "pericytes" of Zimmermann⁴⁷ and Stout,^{35,41,45} the name "perithelioma" is of doubtful value. The suggestion of Bloom³ that these tumors be designated by the noncommittal term "carotid body tumor" seems to be sound.*

Evidence of malignancy was, as noted above, singularly absent in the present series. Most of the tumors were well encapsulated, without evidence of invasion of adjacent structures, and none of the lymph nodes removed showed any tumor.†

It appears that the incidence of malignancy in these tumors may be overemphasized in the literature. I disagree vigorously with the thesis that 50 per cent of these tumors may be classified as malignant on histologic grounds.¹⁷ While it is true that considerable variation in nuclear size, with the presence of giant forms, may occur (Fig. 14), such nuclear changes may be seen in other notably benign tumors, *e.g.*,

* Tumors of the homologous aortic bodies have not been reported in man, but Bloom⁸ described two instances in dogs resembling the carotid body tumors of man. Following the report of Rosenwasser⁴⁰ of a tumor of carotid body type in the middle ear presumably arising from the "glomus jugulare," two other cases have been reported.^{24,31} These tumors may cause extensive destruction of the mastoid bone and petrous ridge.^{24,30} and one has apparently involved lymph nodes.⁴⁶

† The only histologically malignant tumor of possible carotid body origin that I have seen is one sent by Dr. Paul Brindley of the University of Texas. It is a highly anaplastic sarcoma involving the region of the carotid bifurcation and, although its location is compatible with origin from the carotid body, the cells are so poorly differentiated that definite conclusions concerning histogenesis are impossible. In some fields multinucleated giant cells suggestive of myosarcoma are seen. As noted above, middle ear tumors of carotid body type may be at least locally invasive.

parathyroid adenomas. Mitotic figures are certainly the exception rather than the rule, and this is borne out by the frequency of prolonged clinical duration (up to 15 years in the present series).

Not a single case of visceral metastasis has been reported. (The case of Gilford and Davis¹³ does not seem acceptable because of the lack of adequate illustrations.) The possible cerebral metastases recorded by Harrington *et al.*¹⁷ are extremely questionable in the absence of autopsy evidence, and in view of the notoriously high incidence of cerebral sequelae following ligation or damage to the carotid arteries. Histologic evidence is notably lacking also in many cases in which the tumor is said to have recurred locally or to have extended upward to the base of the brain. The rate of postoperative recurrence is stated variously, being usually very low, but in some reviews fairly high.³⁸

It is stated repeatedly in the literature that metastases to regional lymph nodes may occur. When, however, one attempts to find specific instances, the extreme rarity of metastasis becomes apparent. In an extensive, although not exhaustive, search of the literature, I was able to find only 2 cases^{28,29} of histologically verified metastases in lymph nodes. In at least one of these,²⁸ the involved nodes were described as adjacent to the lower pole of the tumor and as being "infiltriert," apparently by direct extension.

The operative mortality is extremely high (about 30 per cent in the present series as well as others), due to the frequent necessity of ligating the carotid arteries in order to remove the tumor. In view of the clinically benign course of most of these tumors and the remarkable absence of symptoms in almost all cases, it would seem inadvisable to attempt surgical removal if it is necessary to ligate the carotids (admittedly it is sometimes impossible to gauge the feasibility of removal without ligation until the operation has progressed to such a point that it must be completed). The warning of Bevan and McCarthy² would seem to be still valid:

"With this evidence [about 30% operative mortality], we wish to present the conclusion that in the future neoplasms of the carotid body should not be removed when it is necessary to ligate the carotid arteries in order to complete the operation. If the common carotid and the internal carotid can be saved by careful dissection, done best under local anaesthesia, the removal of a benign tumor of the carotid gland would be justified. If the surgeon had definite and satisfactory evidence that the tumor was malignant, the huge 30 per cent mortality involved in the ligation of the carotid arteries might be accepted in order to save the patient from death and from malignant disease."

I would add that such "definite and satisfactory" evidence of malignancy is rarely available at the time of operation, or, indeed, subsequently.

SUMMARY

1. According to present evidence, the carotid body is a chemoreceptor, not a gland of internal secretion, and not part of the "chromaffin system."

2. In a series of 17 tumors of the carotid body no true chromaffin reaction was demonstrated, and no evidence for the secretion of epinephrine was obtained in assay of the fresh tissue in two instances.

3. Tumors of the carotid body exhibit a basic pattern of nests of "chief" cells surrounded by a more or less vascular stroma. Depending on the relative amounts of "chief" cells and stroma, they may be described as "usual," "adenoma-like," or "angioma-like."

4. Use of the non-committal term "carotid body tumor" is preferred to other names generally used.

5. The great majority of these tumors are both histologically and clinically benign. In view of the high operative mortality it is doubtful whether they should be removed in those cases in which ligation of the carotid arteries is necessary.

I am indebted to Drs. Shields Warren and Olive Gates for their interest and for making available the material on which this paper is based.

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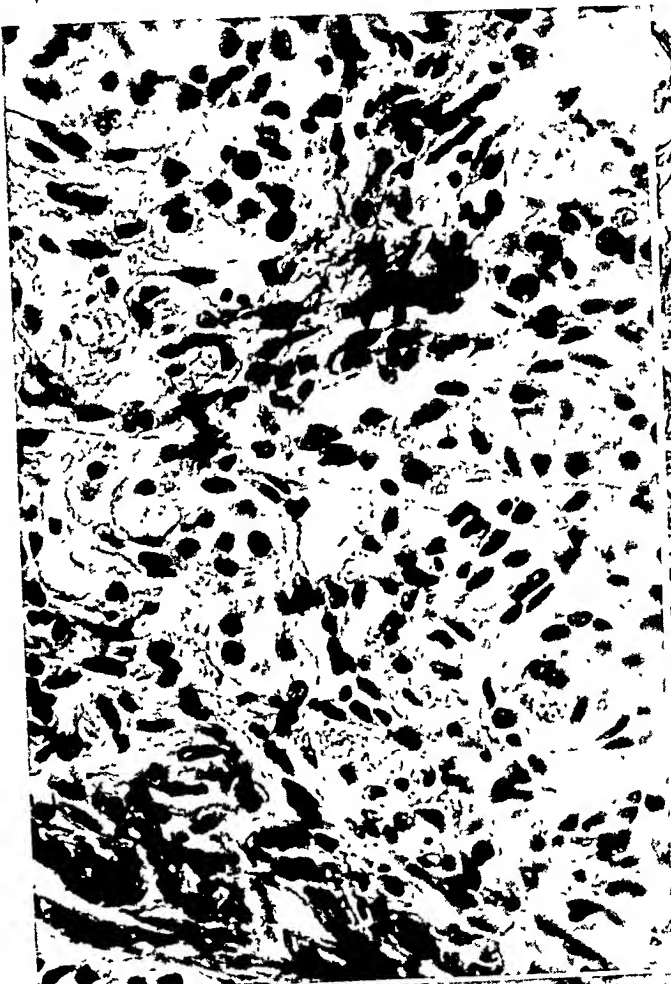
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DESCRIPTION OF PLATES

PLATE 60

- FIG. 1. Normal carotid body. Phosphotungstic acid hematoxylin stain. $\times 400$.
- FIG. 2. Normal carotid body. In this instance the cells are better preserved and plumper than in Figure 1. Hematoxylin and eosin stain. $\times 400$.
- FIG. 3. Normal carotid body. The cell nests or "Zellballen" are clearly brought out. Same carotid body as used for Figure 2. Wilder's silver impregnation for reticulum. $\times 300$.
- FIG. 4. Carotid body tumor. The "usual" pattern, representing a slight exaggeration of the normal structure, with larger cell clusters. Phosphotungstic acid hematoxylin stain. $\times 400$.

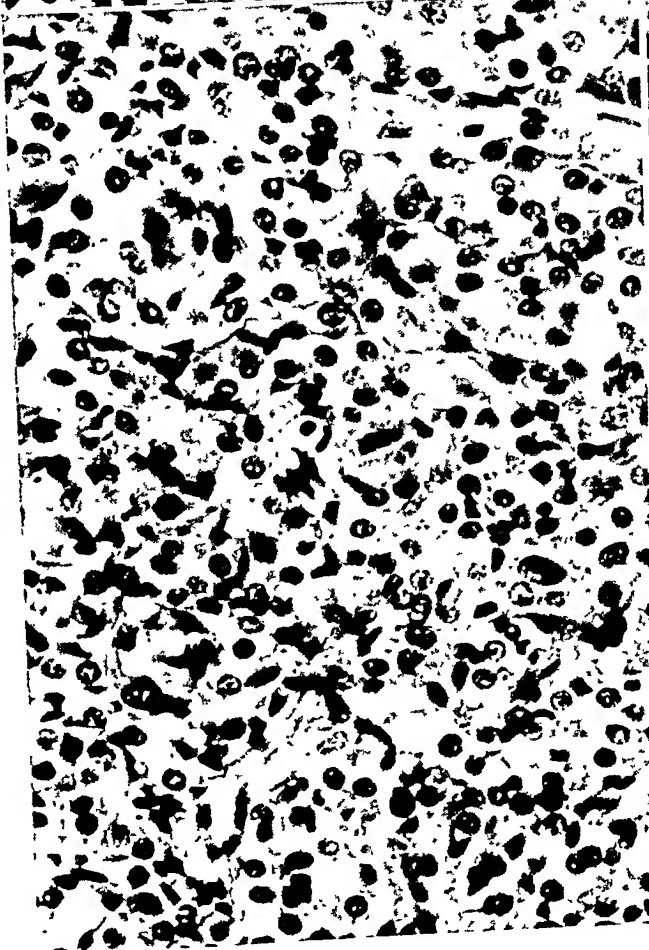
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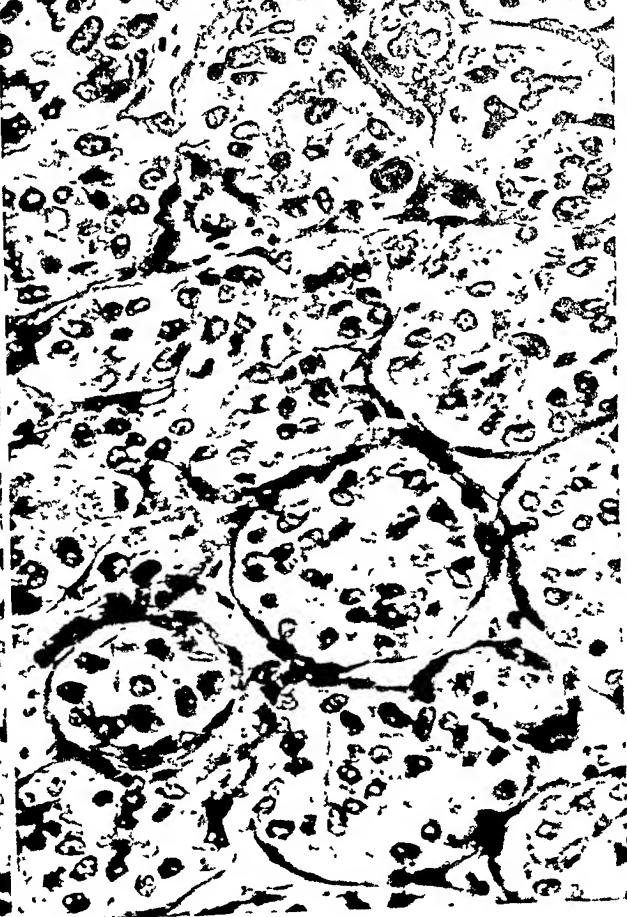


PLATE 61

FIG. 5. Another example of the "usual" type. Phosphotungstic acid hematoxylin stain. $\times 400$.

FIG. 6. The characteristic reticulum pattern of a carotid body tumor. Same case as used for Figure 5. Foot-Bielschowsky stain. $\times 300$.

FIG. 7. A variant of the "usual" pattern with somewhat smaller cell nests. Some of the dark material in the stroma is hemosiderin. Phosphotungstic acid hematoxylin stain. $\times 400$.

FIG. 8. Another variant of the "usual" pattern. Rather poor preservation of the chief cells, with a tendency to elongated shape, perhaps an artifact due to compression. Phosphotungstic acid hematoxylin stain. $\times 400$.

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PLATE 62

FIG. 9. Reticulum pattern of the tumor shown in Figure 8. The stroma is somewhat more abundant than usual. Wilder's silver impregnation. $\times 300$.

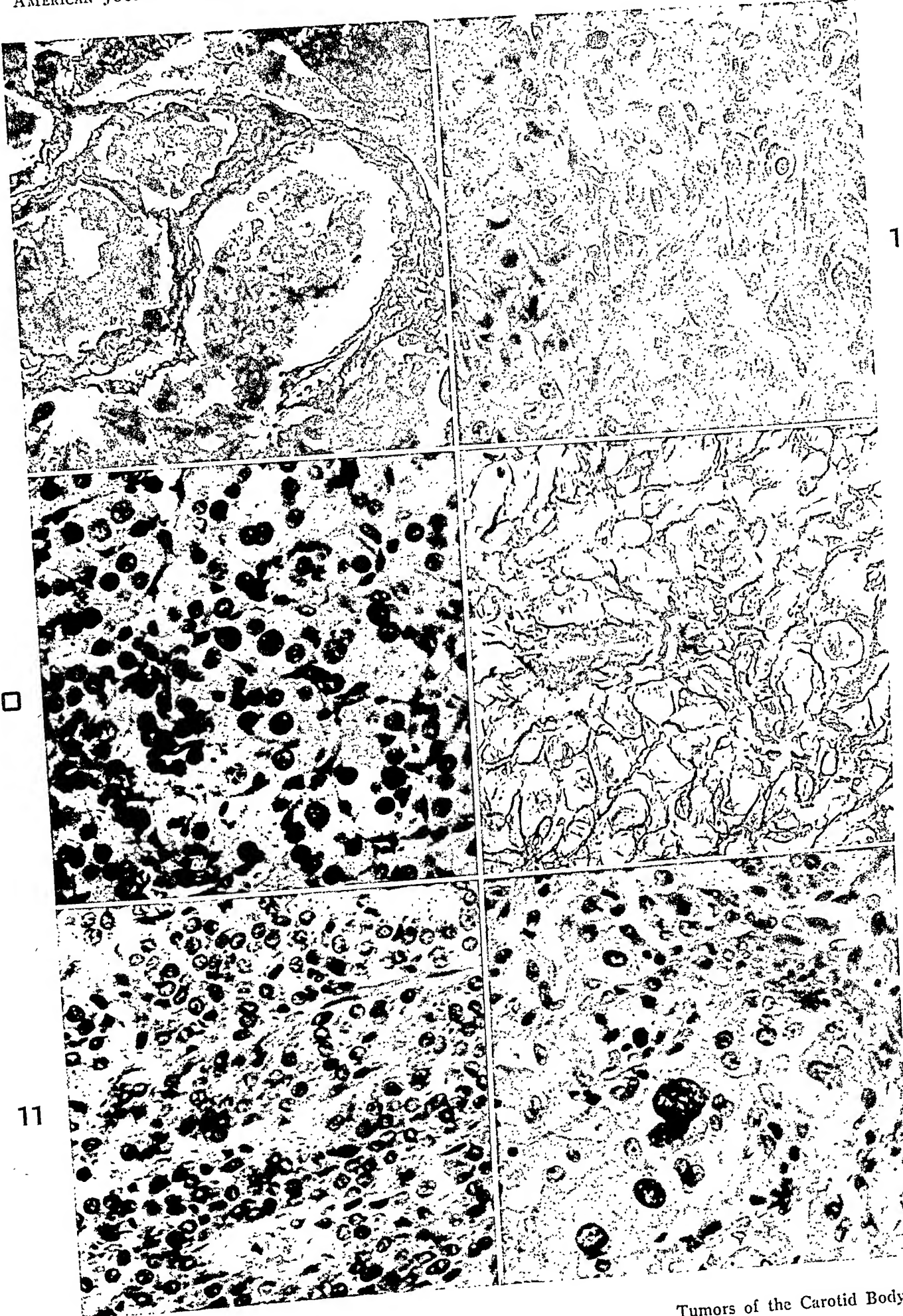
FIG. 10. The "adenoma-like" pattern. The chief cells have a strikingly epithelial appearance, but the arrangement of the "Zellballen" is maintained. Hematoxylin and eosin stain. $\times 400$.

FIG. 11. A variant of the "adenoma-like" type. The chief cells are arranged in large sheets. Phosphotungstic acid hematoxylin stain. $\times 400$.

FIG. 12. The "angioma-like" pattern. Here the chief cells are ovoid or spindle-shaped, and capillaries are numerous. Eosin and methylene blue stain. $\times 400$.

FIG. 13. Silver impregnation (Wilder) of the tumor shown in Figure 12, showing that the fundamental structure is maintained, despite the angiomatous appearance. $\times 300$.

FIG. 14. A tumor showing unusually marked variation in nuclear size, with one giant form. Hematoxylin and eosin stain. $\times 400$.



Tumors of the Carotid Body

PARACOCCIDIOIDAL GRANULOMATOSIS
CARDIAC LOCALIZATION IN A CASE OF 'GENERALIZED FORM *

L. DA CUNHA MOTTA, M.D.

(From the Faculdade de Medicina da Universidade de São Paulo,
São Paulo, Brazil)

Moore¹ stated that "the term blastomycosis in its medical interpretation has come to denote a more or less definite clinical syndrome with a multiplicity of causative agents." However, by reserving the use of the term "blastomycosis" for those mycoses caused by yeast-like cells reproducing in the tissues by budding or gemmation, a great advance would be attained. As Moore said, typical blastomycoses are the infections caused by fungi of the genera *Zymonema*, *Paracoccidioides*, and *Cryptococcus*.

In a broad sense, there is a certain geographic distribution of varieties of the disease, justifying the designations "North American blastomycosis" (caused by the *Blastomyces* or *Zymonema dermatitidis*), "European blastomycosis" (*Cryptococcus neoformans*—*Torula histolytica*), and "South American blastomycosis" (*Paracoccidioides brasiliensis*). However, each of these denominations should be reserved for the prevalent or endemic form of the disease in a particular region, without excluding the possibility of the existence of rare, sporadic cases of the other varieties of blastomycosis.

In Brazil, as well as in other South American countries, the prevalent form of blastomycosis is the disease of Lutz-Splendore-Almeida, produced by the *Paracoccidioides brasiliensis* (Splendore), established by Almeida in 1930. Lutz² (1908) was the first to identify the disease in Brazil, and in the same year Carini³ reported the second case, with the same localization of lesions as in the case of Lutz. In 1909, Splendore started a series of publications on the mycosis of Lutz, and considered⁴ (1912) the causative agent as of the genus *Zymonema* (De Beurmann and Gaugerot), species *brasiliensis*, n.sp.

From then until 1929, there followed a period of incomprehensible disagreement among research workers, during which the chief tendency was to attempt to identify Brazilian blastomycosis with North American coccidioidal granuloma. That tendency is the more surprising when it is considered that Lutz and Splendore described the fungus as reproducing in the tissues by budding. As a matter of fact, in Lutz's first reference the disease was classified as a "pseudococcidioidal" mycosis, differing therefore from the coccidioidal granuloma

* Received for publication, March 26, 1947.

described by Rixford and Gilchrist⁵ in the United States in 1896, the agent of which reproduces characteristically by endosporulation.

The chief works that have contributed to the confusion are as follows: Vianna⁶ stated that he always found parasites multiplying by endosporulation, and only rarely by budding. Carini⁷ described the reproduction of the fungus as being by budding, not endosporulation, and yet concluded inexplicably that the parasite is the *Coccidioides immitis*. Pedroso⁸ was of the opinion that the fungus reproduces by endosporulation, and classified it as *Coccidioides immitis*. Habersfeld⁹ wrote an interesting paper denying peremptorily reproduction by budding for the agent of Brazilian blastomycosis; he supported the view that its only form of reproduction is endosporulation, and classified the fungus as *Zymonema histosporocellularis*. Finally, Fonseca and Area Leão¹⁰ studied one case of Brazilian blastomycosis and described the reproduction of the fungus as being exclusively by endogenous sporulation, the spores being liberated through small openings in the membrane, but continuing to be attached to the mother cell for some time by a protoplasmatic filament. They concluded, therefore, that the fungus is identical to *Coccidioides immitis*, found by Rixford and Gilchrist⁵ in the United States.

In 1927, Souza Campos and Almeida¹¹ were the first to make a comparative study of Brazilian and North American blastomycoses, having had 12 cases of the former and 2 of the latter variety; they were then able to draw conclusions as to their diversity. Proceeding in that comparative study, Almeida¹² established in 1929 that the causative agent of Brazilian blastomycosis is a new species of the genus *Coccidioides*. In 1930, Almeida¹³ finally was able to establish a new genus—*Paracoccidioides*—for that organism, based on abundant material and experimental work, separating it definitely from the genus *Coccidioides*.

Coming to Brazil to study Almeida's new genus, Moore¹ not only confirmed those findings but also described two new species, *Paracoccidioides cerebriformis* and *P. tenuis*.

From the clinical point of view as well as from that of pathogeny, there are fundamental differences between the North and the South American blastomycoses. According to Conant and co-authors,¹⁴ in the North American variety the prevalent localizations are in the skin, lungs, and bones, the lesions being of the suppurative or granulomatous type. The organisms enter the skin or the respiratory mucous membrane. In the Brazilian form, primary cutaneous lesions are exceptional, if existent at all; they usually represent foci of secondary dissemination.¹⁵ In almost all cases, the fungus penetrates the bucco-

pharyngeal mucous membrane,¹⁶ giving rise to painful infiltrative ulcerations, with the papillomatous or vegetative aspect of stomatitis ulcerosa moriformis (mulberry-like)¹⁵ in the gums, floor of the mouth, lips, cheeks, soft palate, uvula, and palatine arches, with occasional extension to pharynx and larynx. The tonsils¹⁶ are usually involved primarily or secondarily with the lesions either visible or not at the surface; the latter circumstance explains some rare cases of cervical paracoccidioid al adenopathy with no apparent lesion in the buccopharynx.

The primary lesions are followed by a rapid lymphatic dissemination with involvement of the cervical lymph nodes. These are gradually enlarged, and the lymphadenitis may evolve to softening and fistulization, or to fibrosis and induration.¹⁷ This lymphatic dissemination may progress to other groups of lymph nodes, namely, the supraclavicular or axillary. This form, with involvement of integument and secondary regional lymphatic dissemination, constitutes the lymphaticotegumental form.* In the great majority of cases these lymphaticotegumental manifestations represent an evolutive phase of the infection, which tends to become systemic by spreading through the blood stream. This may happen after 3 to 18 months, with the development of visceral and cutaneous lesions accompanied by polyadenopathy, leading to cachexia and death.

In the systemic form of visceral generalization any organ may be involved, the more frequently affected being the spleen,¹⁸ liver, intestines,¹⁹ pancreas, and lungs²⁰; since these lesions are accompanied by intense involvement of the regional lymph nodes, this is said to be the lymphaticovisceral form.

Finally, in the terminal stages of the infection the lesions are multiplied through lymphatic and hemal dissemination, constituting the mixed forms, in which the disease spreads to the whole body, and may affect any organ.

REPORT OF CASE AUTOPSY FINDINGS

Autopsy was performed upon the body of a 38-year-old Japanese (height, 151 cm.; weight, 42 kg.) in an advanced state of cachexia, who had had no medical assistance.

The face showed an extensive ulceration of the skin covered by a muddy crust; similar smaller lesions were found in the left eyebrow, lobule of the right ear, lateral aspects of the neck, supraclavicular fossae, axillae, inguinal regions, and thighs. The pharynx, epiglottis.

*Tegumental is here used in its broad sense, that is, "pertaining to the tegument," both cutaneous and mucosal.

and larynx showed multiple irregular ulcerations of various sizes. The tonsils were extensively destroyed. The lungs showed multiple hard whitish areas, especially in the inferior lobes. Hydrothorax (500 cc.) and pleural adhesions were found on the left side. Macroscopically, the heart seemed normal. The abdominal cavity contained about 600 cc. of a turbid serofibrinous exudate, the intestinal loops being covered by deposited fibrin. Several yellowish, hard, miliary nodules were found in the liver, as well as in the spleen and cortical substance of the kidneys. No lesions were observed in the pancreas. The lymph nodes showed lesions varying in size and consistence, but mostly soft caseous areas. The microscopic study of smears of this material revealed the paracoccidioidal fungus in large numbers.

Anatomic Diagnoses. Serofibrinous peritonitis; paracoccidioidal granulomatosis of the tonsils, pharynx, epiglottis, larynx, lungs, liver, spleen, kidneys, lymph nodes, and skin.

Microscopic Findings

Myocardium. Slide 1 (Fig. 1). Inside a myocardial fiber there were a large number of young paracoccidioidal fungi, each measuring 5 to 6 μ in diameter. This conglomerate of small parasites occupied a large part of the length of the muscle fiber and completely destroyed its structure. There was no evidence of adjacent inflammatory reaction. In another field (Fig. 2), there was a single adult fungus inside a muscle fiber, the structure of which was destroyed. Again, no inflammatory reaction was noted in the vicinity.

Slide 2 (Fig. 3). A nodular, poorly limited area was seen in a bundle of muscle fibers, where the structure was altered by the partial destruction of the fibers. The central zone of this area showed a paracoccidioidal fungus in multiple gemmation surrounded by a characteristic marginal crown of spores. Among the tissue débris and around the organism there was a cellular reaction represented by some mononuclear lymphocytoid cells and a few polymorphonuclear leukocytes. This is a later stage of the process seen in slide 1, showing a tendency towards the formation of a nodule of reaction.

Slide 3 (Fig. 4) showed a rarefied, oval, well circumscribed area of destroyed muscle fibers, in which there was some amorphous débris of the fibers and two adult parasites, together with a nodular conglomerate of epithelioid cells, a few lymphocytes, and some polymorphonuclear leukocytes. There were no giant cells.

Slide 4 (Fig. 5) showed an advanced stage of the process. The lesion in the muscle bundle appeared as a rarefied, well circumscribed area of destroyed muscle fibers, in the débris of which there were free

parasites. The area showed also a cellular proliferation represented mainly by histiocytes together with elongated cells having fusiform or oval nuclei, that is, with the characteristics of fibroblasts. There were a few lymphocytes, chiefly at the periphery of the area, but no giant cells.

Slide 5 (Fig. 6). There were two adult fungi, close together, in the interstitial tissue between two muscle bundles. In close contact with the capsule of the parasites there were several elongated nuclei, and in their immediate vicinity there was a moderate cellular reaction represented by lymphocytoid elements and a few fibroblasts.

Epicardium. In the epicardium two cellular nodules were found containing fungi and formed by a reactionary proliferation of histiocytic mononuclear cells and small lymphoplasmacytic elements. In one of the nodules, around the parasite, a poorly limited acidophilic mass was seen containing four small adjoining nuclei, suggesting the formation of a giant cell. Both nodules were deeply situated in the serosa, close to the myocardium. In the lumen of an epicardial vessel (Fig. 7), several paracoccidoidal fungi were seen free in the circulating blood; one of these was in the process of reproduction.

Lungs. The lungs showed nodular structures in the alveolar walls, projecting to various degrees into the lumina of the alveoli. The structures were constituted by a proliferation of histiocytes, one or more giant cells, lymphocytes, and plasmacytes. As a rule, the nodules contained large numbers of parasites, the majority of which were phagocytized; many of them were in reproduction. Close by, in the interstitial tissue, there was a similar granuloma at the side of a vessel. In other fields the alveolar lumina were free, while the walls were thickened by a marked dilatation of the congested capillaries. In neighboring areas the alveolar lumina were occupied by a homogeneous acidophilic substance containing erythrocytes and large, rounded mononuclear cells. In this massive zone, some irregularly distributed alveolar lumina contained a cellular conglomerate of large epithelioid cells together with one or more multinucleated giant cells phagocytizing parasites, some of which were in reproduction.

Lymph Nodes. The normal structure of the lymph nodes was profoundly altered. There were extensive irregular areas, isolated or confluent, formed by poorly stained debris of disintegrated tissue, containing a large quantity of free parasites, some of which were in reproduction. There remained only a few small conglomerates of lymphocytes and lymphadenoid tissue, mostly at the periphery of the node, where small granulomas were found containing parasites included in giant cells and histiocytes.

Liver. Numerous well circumscribed granulomas of various sizes were disseminated throughout the liver. They consisted of epithelioid histiocytes, a few lymphocytes, and, in most cases, one or more giant cells phagocytizing fungi, the majority of which were in reproduction. Some of the granulomas showed an incipient fibrosis, revealed by nuclei of fibroblasts found at the periphery or inside the nodule. The granulomas were localized both inside the hepatic lobules and in the stroma of the spaces of Kiernan.

Spleen. The focal lesions of the spleen were found both in the lymphatic nodules and splenic pulp. Most of the malpighian bodies showed variable disorganization in their constitutive elements, which were dissociated by nodular granulomatous proliferations. The histiocytic reaction in the red pulp was extensive, and included giant cells with phagocytized fungi as well as numerous plasmacytes and a fair number of eosinophils.

Pancreas. The structure of the pancreas was preserved, including the islands of Langerhans. There was a slight increase in the interstitial connective tissue. A few nodular granulomas were found in the lobules (Fig. 8), showing a slight histiocytic reaction and some giant cells phagocytizing parasites.

Kidneys. Lesions were found both in the cortical and medullary substance of the kidneys. Several intact glomeruli showed a small number of free parasites in the lumina of the capillary loops. In Figure 9 an organism is seen entering the afferent arteriole. When the number of parasites was high, the glomeruli showed varying degrees of necrosis, due to direct toxic action of the fungi; when that number was low, the glomeruli remained intact, and no periglomerular reaction was seen (Fig. 10). In the damaged glomeruli Bowman's capsule was partially destroyed and there developed a periglomerular reaction with numerous lymphocytes, some neutrophils, and mononuclear cells. This exudate sometimes included a parasite which had migrated through the ruptured Bowman's capsule (Figs. 11 and 12). The lumina of the convoluted and straight tubules showed varying numbers of parasites. When their number was low, there was no reaction in the vicinity; but when fungi were numerous (sometimes they filled the tubular lumen) the wall might be destroyed, and there was an adjacent inflammatory reaction similar to the one observed around the damaged glomeruli (elimination form).

The preparations also showed disseminated areas of variable size, mostly in the cortical substance where the normal structure was replaced by a granulomatous tissue, representing a more advanced stage of the process. They consisted of a proliferation of histiocytes, numer-

ous giant cells with phagocytized parasites, lymphocytes, plasmacytes, and some eosinophils, found mostly in the peripheral zone of the lesion. In some of these areas the core was occupied by densely packed neutrophils, the majority of them degenerated, while in other lesions the central portion was invaded by fibroblasts.

Skin. The skin showed two distinct lesions. One was localized in the dermis, extending out to the papillary layer, and consisted of two juxtaposed nodular areas. These areas showed a dense conglomerate of parasites destroying the pre-existing structures, surrounded by a coat of parvicellular elements, predominantly plasmacytes. Many of these cells showed regressive alterations of their nuclei, mostly pyknosis. The adjacent vessels and glands were densely infiltrated, mostly by plasmacytes. The covering epidermis showed no alterations.

The other lesion was more extensive. It was situated in the dermis and expanded secondarily to the epidermis. In the deeper coat it was similar to the first lesion. In the epidermis, the basal and spinous strata were destroyed, while the stratum corneum showed a marked parakeratosis and invasion of paracoccidioid fungi, neutrophils and, at the surface, colonies of bacteria. The neutrophils extended secondarily to the dermis, chiefly to the papillary layer.

Diagnosis

Paracoccidioid myocarditis, pericarditis, bronchopneumonia and interstitial pneumonitis, lymphadenitis (gummatous form), hepatic granulomatosis (nodular form), splenic granulomatosis (nodular and disseminate forms), pancreatic granulomatosis, hematogenic nephritis, and dermatitis with secondary ulceration.

DISCUSSION

The case described presents a condition of acute paracoccidioid sepsis, as a terminal stage of a paracoccidioid granulomatosis. As far as could be ascertained, this is the first demonstration in histologic sections of the reproduction of paracoccidioid fungi in the circulating blood. Besides the consequent localization of the organisms in several organs, as far as is known this is also the first report of cardiac lesions caused by this fungus.

In this case, the dissemination to the heart was hematogenic. Initially, the parasites may enter the myocardial fibers or the interfascicular connective tissue. In the former localization the parasite was found in the adult stage, as well as in its small, young forms. In both localizations there is a profound structural alteration of the muscle fiber, with destruction of myofibrils through compression or toxic action of the

parasites. This stage shows, as yet, no local inflammatory reaction to the presence of the organisms, as they are still inside the fiber and do not activate the adjacent tissues. To this initial direct lesion of regressive character there is added the destruction of the muscle fiber and liberation of organisms and debris in the interstitial tissues, where they give rise to a local reaction. This initiates the appearance of lymphocytoid cells and a few neutrophils.

In subsequent stages, mononuclear and histiocytic cells of epithelioid aspect appear and predominate; their local conglomeration constitutes the granulomatous nodule, which usually contains parasites. In some granulomas, a marginal fibroblastic proliferation develops, indicating the tendency to sclerosis of the lesion. The death of the patient possibly interrupted the progress of the process to the later stages that sometimes have been found in other organs of other patients.

The interstitial and epicardial lesions develop in a like manner.

The renal lesions reached a higher development than the ones just referred to. The dissemination to the kidney is also hematogenic, as is well evidenced in Figure 9, where the parasite is entering the glomerulus through an afferent arteriole. In the glomeruli, the fungi induce regressive lesions and necrosis of varying portions of the capillary loops, or even total destruction of the glomerulus. The lesion of the Bowman's capsule opens the way to an active periglomerular interstitial reaction. The periglomerulitis consists of a dense conglomeration of lymphocytes, monocytes, and neutrophils. Through the destruction of glomerular capillaries the fungi enter Bowman's space and are drained to the renal tubules, where they give rise to new foci, thus characterizing the form of elimination of hematogenic nephritis.

All of the multiple lesions found in the different organs show the characteristics of an acute hematogenic dissemination, including the cutaneous lesions, which arose in the dermis.

SUMMARY

A case of paracoccidioidal granulomatosis (Brazilian blastomycosis) is reported, terminating in an acute paracoccidioidal sepsis.

The findings may be summarized as follows:

1. *Paracoccidioides brasiliensis* was found reproducing in the circulating blood.
2. Specific myocardial and epicardial lesions are reported for the first time.
3. The renal lesions are of the hematogenic focal nephritic type.
4. The multiple lesions found in several organs, including the skin, demonstrate the acute hematogenic dissemination of the infection.

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[Illustrations follow]

DESCRIPTION OF PLATES

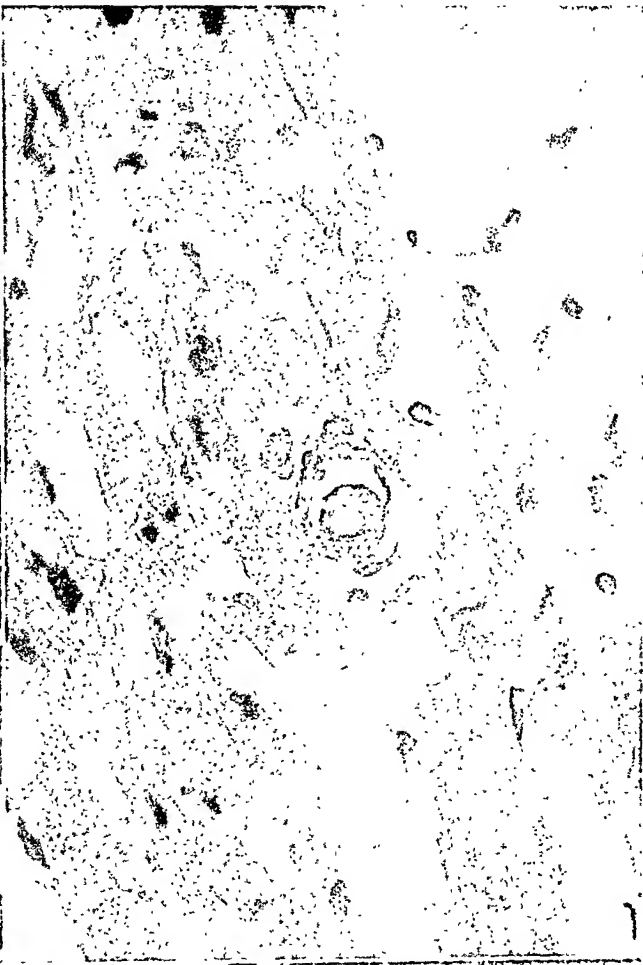
PLATE 63

- FIG. 1. Young paracoccidioidal fungi inside a myocardial fiber. Hematoxylin and eosin stain. $\times 950$.
- FIG. 2. Adult fungus inside a myocardial fiber. Hematoxylin and eosin stain. $\times 750$.
- FIG. 3. Early nodular reaction around a paracoccidioidal organism in multiple gemmation, in the myocardium. Hematoxylin and eosin stain. $\times 750$.
- FIG. 4. Nodular area of reaction containing two adult parasites, and showing destruction of the myocardial fibers, epithelioid cells, lymphocytes, and some polymorphonuclear leukocytes. Hematoxylin and eosin stain. $\times 750$.

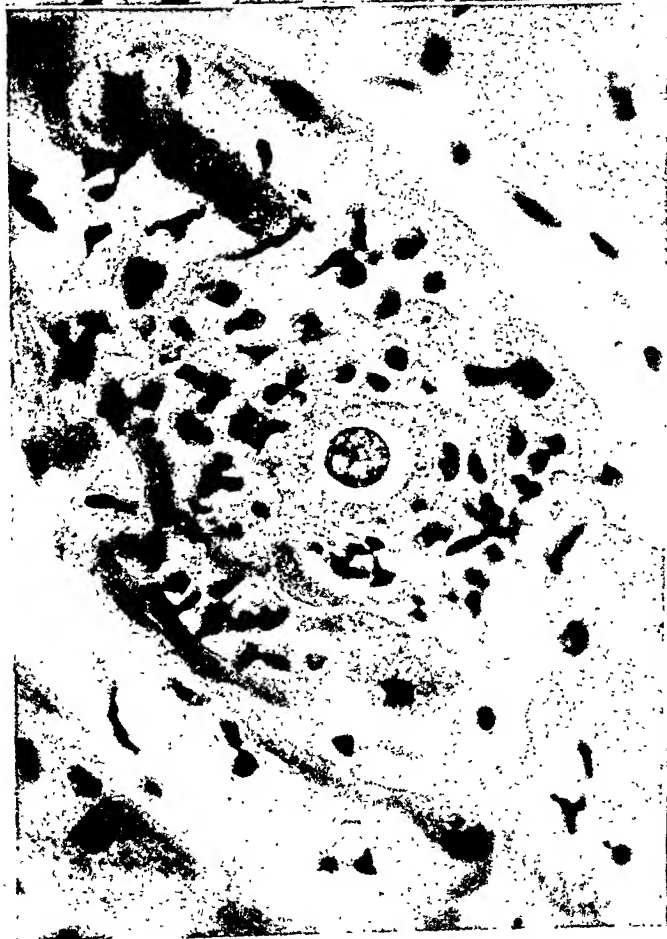
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4



PLATE 64

FIG. 5. Advanced stage of the process in a muscle bundle, showing destruction of myocardial fibers, free parasites, histiocytes, and initial proliferation of fibroblasts. Hematoxylin and eosin stain. $\times 450$.

FIG. 6. Two adult parasites in the interstitial tissue of the myocardium. Hematoxylin and eosin stain. $\times 450$.

FIG. 7. *Paracoccidioides* in reproduction, free in the circulating blood of an epicardial vessel. Hematoxylin and eosin stain. $\times 950$.

FIG. 8. Granuloma in the pancreas, containing a parasite in reproduction. Hematoxylin and eosin stain. $\times 185$.

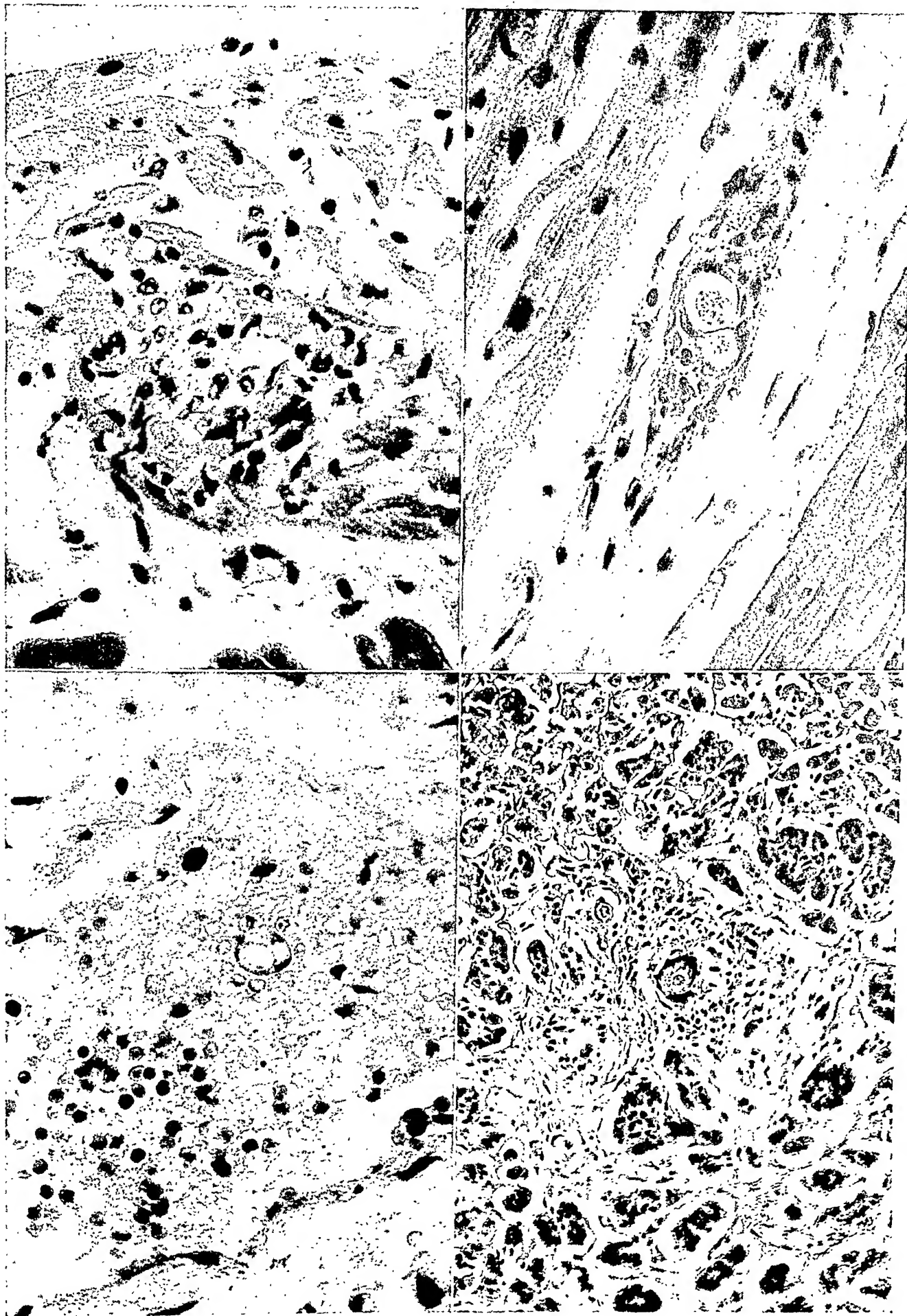


PLATE 65

FIG. 9. *Paracoccidioides* free in the blood of an afferent glomerular arteriole. Hematoxylin and eosin stain. $\times 500$.

FIG. 10. Single parasite in a glomerulus. No periglomerular reaction is seen. Hematoxylin and eosin stain. $\times 500$.

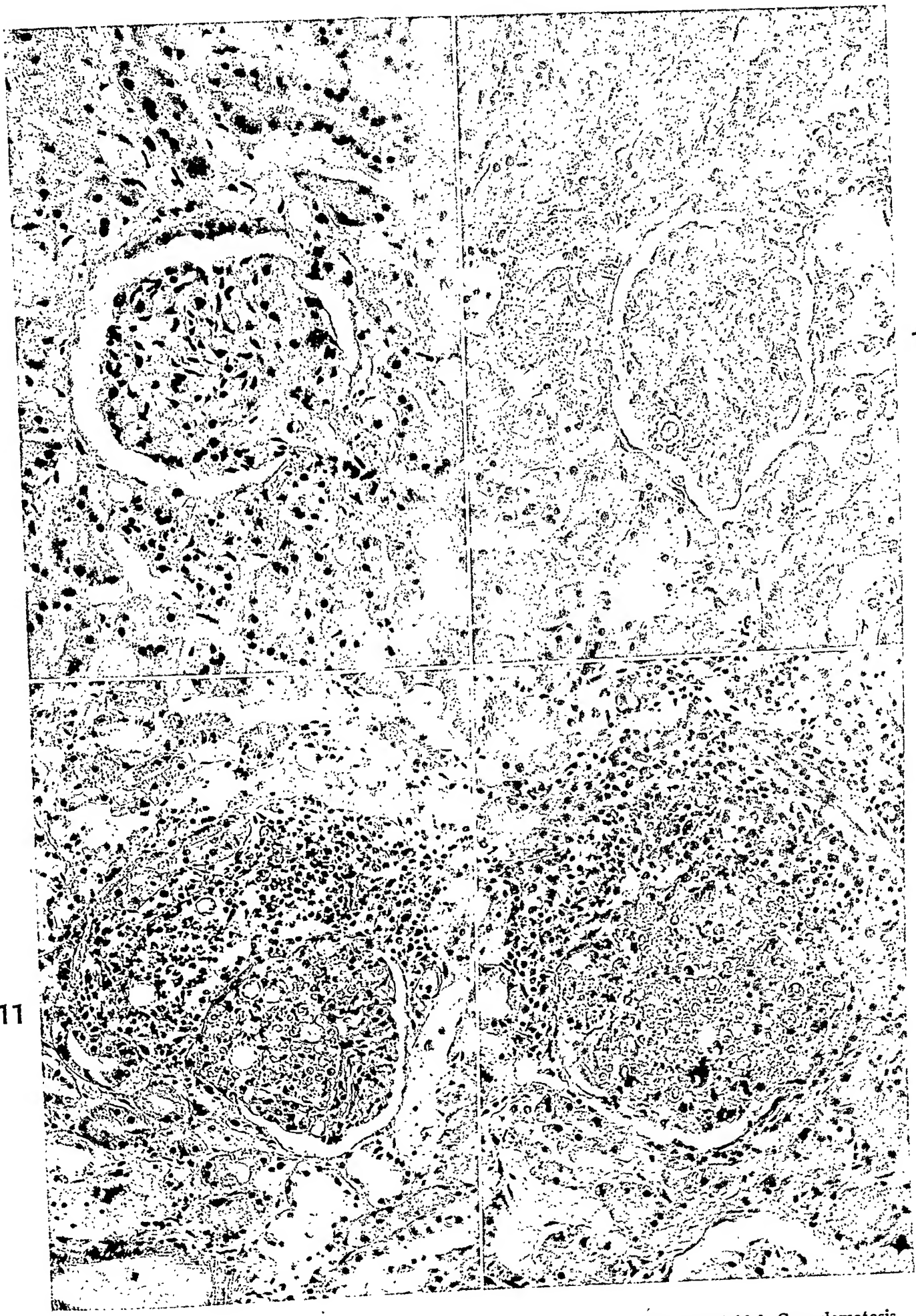
FIGS. 11 and 12. Glomerulus with numerous parasites, showing rupture of Bowman's capsule with periglomerular reaction. In the exudate there are also parasites migrating through the ruptured Bowman's capsule. Hematoxylin and eosin stain. $\times 230$.

9

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12

11



Paracoccidioid Granulomatosis.

SYSTEMIC NORTH AMERICAN BLASTOMYCOSIS
REPORT OF A CASE WITH CULTURAL STUDIES OF THE ETIOLOGIC AGENT
AND OBSERVATIONS ON THE EFFECT OF STREPTOMYCIN AND PENICILLIN
IN VITRO *

M. L. LITTMAN, Ph.D., EUGENE H. WICKER, M.D., AND ALBERT S. WARREN, M.D.
(From the Department of Pathology and Bacteriology and the Department of Internal
Medicine, School of Medicine, Tulane University of Louisiana, and the Charity Hospital
of Louisiana, New Orleans, La.)

North American blastomycosis is a fungus disease caused by *Blastomyces dermatitidis*. It is manifested in two clinical forms, cutaneous and systemic. The former, beginning as a small pimple or pustule on the hands, face, wrists, ankles, or other exposed site, or in an area injured by abrasion, proceeds to a chronic or subacute ulcerating lesion of the skin. Skin lesions of cutaneous blastomycosis are characteristically single, spread slowly and peripherally, and may cause extensive involvement with no evidence of visceral infection. The cutaneous form of the disease is seldom fatal and usually responds to treatment with iodides and x-ray therapy. Systemic blastomycosis, on the other hand, is a fatal disease ordinarily characterized by pulmonary involvement and widely disseminated lesions in the subcutaneous tissues, bones, joints, central nervous system, and internal organs.¹ In contrast with the cutaneous type, skin lesions in this form of the disease are multiple instead of single and are widely distributed over the skin on unexposed parts of the body such as the abdomen, back, and thighs. Both cutaneous and systemic manifestations of blastomycosis are caused by the same fungus.

The characteristic reaction of the tissues to the organism in either systemic or cutaneous blastomycosis is abscess formation with chronic granulomatous inflammation, giant cells, necrosis, and fibrosis. Numerous abscesses of almost microscopic size are found in skin lesions, while old lesions show signs of chronic inflammation and scarring. There is extensive epithelial hyperplasia about the margins of the ulcerating skin lesions. Although the tissue reaction in the systemic form of the disease is usually pyogenic, the presence of caseation necrosis and tubercle formation may cause it to be confused with tuberculosis.² Nevertheless, the tissue reactions in blastomycosis and tuberculosis are not identical,³ the most obvious difference being the suppurative reaction in blastomycosis. Comprehensive reviews of the subject are available in the excellent papers of Martin and Smith,⁴ of Baker,⁵ and

* Received for publication, May 24, 1947.

of Friedman and Signorelli.⁵ Cases of North American blastomycosis have been reported almost exclusively from the United States. Proved and presumptive cases have been found in 28 states, with Illinois, Louisiana, Kentucky, Tennessee, Virginia, and North Carolina furnishing the larger numbers.

The purposes of the following report are (1) to present the clinical and pathologic features of a case of fatal systemic North American blastomycosis involving the meninges as well as other organs of the body; (2) to present and discuss the method of primary isolation and identification of *B. dermatitidis*; and (3) to describe tests of the resistance of the fungus *in vitro* to streptomycin and penicillin.

REPORT OF CASE

A 20-year-old, colored, male laborer entered Charity Hospital on May 16, 1946, because of tender cutaneous nodules which had grown progressively larger and more painful over the preceding 3 weeks. He had been well prior to the present illness and had had no symptoms referable to the gastrointestinal, genito-urinary, or nervous systems. For a period of 3 months he had had slight afternoon fever, night sweats, and blood-streaked sputum. He had been employed as a laborer in an oil field in central Mississippi but reported no prolonged contact with irritating dusts or chemicals. The past history was otherwise noncontributory.

Physical Examination. The patient was lying flat in bed without dyspnea, and was oriented and alert; his respiratory rate was 23; pulse, 94; and temperature, 99.4° F. He was well developed but poorly nourished and appeared severely ill. On the left forehead there was a large encrusted lesion, measuring 2 by 3 cm., which was sharply demarcated from the surrounding normal skin. Pus could be expressed from beneath the eschar. There were no lesions of the mucous membranes. Multiple subcutaneous, fluctuant masses, painful to palpation and varying in diameter from 2 to 5 cm., were present over the arms, forearms, thighs, and legs. There was no palpable enlargement of the superficial lymph nodes. The lungs were clear to percussion and auscultation. The heart was not enlarged and no abnormal sounds were heard. There were no palpably enlarged viscera in the abdomen and no abdominal tenderness or rigidity. There was no edema of the extremities. No signs of meningismus were elicited.

Laboratory Findings (on admission). *Blood:* Hemoglobin, 7 gm. per 100 cc.; red blood cells, 2.75 million per cmm.; white blood cells, 15,350 per cmm. Differential blood cell count: 63 per cent polymorphonuclear leukocytes, 13 per cent immature polymorphonuclear leukocytes, 4 per cent monocytes, 20 per cent lymphocytes. Sedimentation rate (Wintrobe) was 67 mm. in 20 minutes, 78 mm. in 1 hour. The Mantoux test was negative with 1:10,000 dilution of old tuberculin. The Kline and Kolmer tests were negative. *Urine (voided specimen):* Yellow, acid; specific gravity, 1.012; albumin, negative; sugar, negative; 2 to 3 red blood cells and 5 to 6 white blood cells per high power field.

Clinical Course. A roentgenogram of the chest taken on admission revealed multiple infiltrations of pin-head size throughout both lung fields (Fig. 1). Since the findings were compatible with miliary tuberculosis, the patient was admitted directly to the Tuberculosis Unit. Sputum examinations (24-hour concentrations) on three occasions revealed no acid-fast bacilli. The vital capacity was recorded as 1,600 cc. The patient was febrile with sharp, irregular fluctuations in temperature up to 102° F. He showed severe muscular weakness throughout the period of observation and frequently required codeine for relief of chest pain.

Toward the end of the first hospital week in the Tuberculosis Unit, the patient became irrational and it was feared that he had developed tuberculous meningitis. A spinal puncture yielded clear cerebrospinal fluid under an initial pressure of 210 mm. of water. The fluid contained 10 cells per cmm., and 31 mg. per cent of protein. The Kline and Kolmer tests were negative; the colloidal gold test gave no reaction.

Cultures of sputum collected on May 24 showed growth of fungi after 6 days, but the organisms were not definitely identified. Pus was then aspirated from the subcutaneous abscesses and examined microscopically in a moist mount. Numerous doubly contoured bodies, measuring approximately 6 to 16 μ in diameter, were seen. Several of the organisms possessed single buds extending from the periphery of the cell. A diagnosis of blastomycosis was entertained and on the 18th hospital day the patient was transferred to the Isolation Unit for treatment.

Upon admission to the Isolation Unit, the patient was anemic, emaciated, edematous, dyspneic, and irrational. Crepitant râles and dullness were present in the base of the left lung and in the left axilla. The heart rate was rapid but regular and a systolic murmur was heard at the apex. The liver was tender and extended 3 finger-breadths below the costal margin. No other masses were felt in the abdomen. The deep reflexes were active and equal. Nuchal rigidity was present, as was Kernig's sign. The subcutaneous abscesses were still present and were quite painful. Microscopic examination of pus from the ulcer on the forehead revealed many oval, doubly contoured, highly refractile bodies which developed small peripheral buds when left at room temperature for 24 hours on a sealed slide mount.

The patient was given a high-vitamin, soft diet, and sedation as needed. He required increasing amounts of narcotics to control the pain in the chest. A transfusion of 500 cc. of whole blood and repeated infusions of normal saline and glucose solution were administered. However, he failed rapidly. His respiratory rate increased to 52 per minute and he became more irrational, experiencing visual and auditory hallucinations during the last 2 days of life. He gradually lapsed into coma, refused all food, and expired on the 22nd hospital day, approximately 4 months after the onset of symptoms.

Clinical Diagnosis: Systemic blastomycosis.

Autopsy Report

Autopsy was performed 2½ hours after death. The body was that of a well developed, poorly nourished, colored, adult male weighing 120 lbs. and measuring 177 cm. in length. Marked pedal edema was present. Granulating ulcers of the skin with slightly raised, irregular edges and necrotic, hemorrhagic bases were present as follows: lesion, 3 cm. in diameter, on the left side of the forehead; two lesions, 3 and 1.5 cm. in diameter, respectively, on the right side of the chin; lesion, 1 cm. in diameter, under the chin; lesion on the right hip, 3 by 1 cm. in diameter. Small, firm, cervical lymph nodes were palpable on the left side of the neck. Round to oval subcutaneous abscesses varying from 3 to 8 cm. in diameter were noted in the following sites: the left side of the neck below the angle of the jaw, the midportion of the inner aspect of the right forearm, below the elbow on the medial aspect of the left forearm, and the calf of the left leg. The pus in these cavities was creamy and varied from pale yellow to greenish gray or bloody. A draining sinus, 1 cm. in diameter, was present in the thoracic wall

in the 4th intercostal space about 1 cm. to the right of the sternum. A necrotic portion of the 4th rib was exposed in the depths of this sinus but there was no communication with the pleural cavity.

The peritoneal surfaces were smooth and shiny. The cavity contained about 500 cc. of clear yellow fluid. The liver extended 4 cm. below the xiphoid and 3 cm. below the costal margins. The abdominal viscera were grossly normal. The mesenteric lymph nodes were not enlarged. The pleural cavities contained no free fluid. The surfaces of the lungs were smooth and shiny anteriorly, but posteriorly the pleural space on the right was obliterated by firm, fibrous adhesions which also involved the diaphragmatic pleura. An abscess, 2 cm. in diameter, was present in the intercostal muscles of the second interspace just to the right of the sternum. The abscess did not communicate with the skin or pleural cavities but had almost ruptured through the parietal pleura. It was filled with thick, yellow-green pus. The pericardial cavity appeared normal. The heart weighed 200 gm. and appeared to be normal. The lungs together weighed 2,490 gm. Innumerable small, shiny nodules, measuring 2 to 3 mm. in diameter, were visible and palpable beneath the pleura of all surfaces of both lungs. Similar discrete nodules were found throughout the substances of both lungs (Fig. 2). On section, the nodules were firm and yellow without obvious central liquefaction. All lobes were rubbery, subcrepitant, and congested. The hilar and mediastinal lymph nodes were moderately enlarged, firm, and pink. The bronchi were filled with red, frothy fluid. The spleen weighed 220 gm. On section, the parenchyma was reddish purple and studded throughout with firm, yellow nodules resembling those in the lungs. The liver weighed 1,660 gm. Scattered small, yellow nodules were present beneath the capsule but were not found elsewhere in the parenchyma of the liver. The right and left kidneys weighed 176 and 180 gm., respectively. The cortical surfaces were smooth, reddish brown, and were elevated by numerous firm, yellowish nodules similar to those already described. On section, the cortices were similarly studded with firm, yellow nodules measuring up to 8 mm. in diameter. The prostate gland was slightly enlarged and soft. On section an abscess, 0.8 cm. in diameter, was present in the right lateral lobe. Its cavity was filled with thick, yellow pus. The gastrointestinal tract and the remaining viscera were of normal appearance.

The brain weighed 1,330 gm. It was soft and edematous, the gyri were flattened, and the sulci narrowed. The dura mater was not thickened or otherwise abnormal except in a region 3 cm. wide running along the superior sagittal sinus for a distance of 5 cm. in the occipital region. In this region, the under surface of the dura was tenaciously

adherent to a semi-necrotic mass of friable, yellow tissue, 2 to 12 mm. thick, which spread laterally about 3 cm. on either side of the sinus and extended down between the occipital lobes along the falx cerebri to its inferior margin. The lining of the superior sagittal sinus in this region was roughened and granular but the lumen was not occluded. An attempt was made to lift the dura and the adherent necrotic material from the brain surface, but no clear line of demarcation was present. Necrosis extended into the brain to a depth of several mm. over the medial and superior surfaces of the parietal and occipital lobes (Fig. 4). The leptomeninges of the base of the brain, cerebellum, pons, and medulla were covered by a tenacious, thick, yellow-green exudate, while the meninges covering the convexities of the cerebral hemispheres appeared normal. The vessels of the leptomeninges were moderately congested.

Microscopic Examination

The heart was histologically normal except for slight edema of the pericardium.

The lungs showed numerous uniformly distributed focal areas of necrosis and suppuration, each surrounded by a granulomatous zone. These granulomata varied greatly in size, the larger ones corresponding to the nodules seen grossly. The granulomata occupied about one-half of the area of the sections examined; some were poorly delimited and others were surrounded by a zone of fibrous tissue. They consisted of necrotic debris, polymorphonuclear neutrophils, epithelioid cells, and round or ovoid yeast-like bodies measuring 8 to 12 μ in diameter. The yeast-like bodies had a highly refractile, doubly contoured cell wall surrounding a granular, central, cytoplasmic mass. Many large multinucleated giant cells were present chiefly at the periphery of the granulomata and some of them contained yeast-like bodies in their cytoplasm (Fig. 3). The alveolar architecture was destroyed within the granulomata but the intervening pulmonary tissue showed remarkably little change except for edema fluid in occasional alveoli.

The spleen contained focal granulomatous and necrotic areas similar to those in the lungs. Some of these lesions contained few, and others many, yeast-like organisms, either free or within giant cells. The intervening splenic pulp was normal except for moderate fibrosis. The malpighian bodies were well preserved and were seldom involved.

The liver showed widely scattered, small, granulomatous lesions containing yeast-like bodies, but was otherwise normal. The gallbladder, pancreas, gastrointestinal tract, and adrenals were normal.

The kidneys contained sharply delimited regions of necrosis, granu-

lomatous inflammation, and suppuration. Many yeast-like organisms were present in these lesions, both free and within the cytoplasm of giant cells. There were degenerative changes of the epithelium of the convoluted tubules and occasional hyaline and granular casts in tubular lumina. The urinary bladder and testes were normal.

The prostate contained multiple abscesses. Many yeast-like organisms were present in the abscess cavities, in the neighboring pus-filled glands, and in the cytoplasm of large multinucleated giant cells.

The mediastinal lymph nodes showed diffuse infiltration with polymorphonuclear neutrophils and marked interstitial fibrosis. Many yeast-like bodies and giant cells were present.

The skin from the lesions on the forehead showed extensive fibrosis of the dermis and subcutaneous tissues. There were many irregular regions of suppuration and granulomatous inflammation surrounded by dense fibrous tissue. Within these regions yeast-like bodies were present, both free and within giant cells.

In the right occipital lobe of the brain and near the midline, the superficial layers of the cortex were destroyed and replaced by granulation tissue and inflammatory exudate. The exudate contained many polymorphonuclear neutrophils together with lymphocytes, plasma cells, and macrophages. Scattered throughout were numerous round, doubly contoured yeast-like bodies, varying in diameter from 6.4 to 12.3 μ . Some of these showed budding (Fig. 11). The cortical tissue beneath the region of superficial destruction showed marked edema and degenerative changes of nerve cells. Gliosis was not apparent. The spaces of the overlying leptomeninges were filled with similar exudate containing many yeast-like organisms. In neighboring regions the meninges contained inflammatory exudate but there was no destruction of the underlying cortex. Sections from several other regions showed no inflammatory changes in the meninges or in the brain.

The cerebellum showed extensive superficial destruction and inflammatory changes of the meninges similar to those described. The cerebral peduncles, pons, and basal ganglia were normal. Extensive inflammatory changes were present in the leptomeninges covering the medulla, and numerous yeast-like bodies were found.

A mass of granulomatous tissue exceeding 1 cm. in thickness was attached to the internal surface of the dura mater in the region of the falx cerebri. Much of this mass was completely necrotic, but in many regions innumerable yeast-like bodies were present, mingled with giant cells, polymorphonuclear neutrophils, macrophages, plasma cells, and lymphocytes. Such collections were in many instances completely surrounded by collagenous fibrous tissue. New blood vessels and fibro-

blasts were present except in regions of necrosis and suppuration. The superior sagittal sinus was almost completely surrounded by tissue of this character and in one region the wall was destroyed. Granulation tissue containing many organisms had replaced the wall of the sinus in this region and had encroached on the lumen.

Anatomic Diagnosis. Systemic blastomycosis with involvement of the skin, subcutaneous tissues, lungs, liver, spleen, kidneys, prostate, bone, meninges, superior sagittal sinus, and brain.

Summary of Pathologic Findings. This case of systemic blastomycosis was characterized by widespread abscesses and granulomatous foci in which the polymorphonuclear neutrophil was the predominating cell. In all of these lesions there were numerous round, oval, yeast-like bodies, either free or within giant cells, measuring 4.6 to 14.0 μ in diameter, which resembled *B. dermatitidis*. Lesions were found in the lungs, liver, spleen, and kidneys. In addition, there were cutaneous lesions of the face which lacked the typical gross appearance of blastomycotic ulcers but nevertheless contained organisms. Subcutaneous abscesses and lesions of the ribs, brain, and meninges were present also. Grossly and microscopically, the lesions in the lungs bore some resemblance to miliary tubercles, but were more like miliary abscesses in that the granulomatous zones were heavily infiltrated with polymorphonuclear neutrophils and their centers were suppurative rather than caseous. The histologic characteristics of the lesions in this case were similar to those described by Baker.³

PRIMARY ISOLATION AND IDENTIFICATION OF THE FUNGUS

The method of primary isolation of the fungus was similar to those recommended by Benham⁶ and Martin and Smith.⁷ Direct examination of exudates was made by means of unstained moist mounts, in saline solution and in 10 to 20 per cent NaOH, and by Wright's staining. Numerous spherical, doubly contoured, thick-walled, yeast-like cells were seen. Exudate was streaked by the gridiron technic on agar plates containing 5 per cent sheep's blood with heart infusion base and Sabouraud's dextrose agar (pH 5.6). Incubation was at 37° C. and room temperature, respectively. Growth on Sabouraud's agar, at room temperature, was not apparent until 7 days after inoculation, when many small colonies of a white, filamentous mold appeared along the lines of streaking.

Growth at 37° C. Growth appeared on blood agar on the fourth day of incubation in the form of numerous well isolated, tiny, filamentous, fungus colonies growing along the lines of streaking. Transfer of one colony from this plate to a blood agar slant resulted in the development

4 days later of a mealy, adherent growth resembling the growth of *Mycobacterium tuberculosis* on glycerin media. A giant colony of this type is shown in Figure 5. Microscopic examination of the growth revealed numerous large, round, thick-walled, doubly contoured, single or budding cells, 5.7 to 16.4 μ in diameter, and thin, segmented mycelial strands (Fig. 7). Upon second and third serial transfers to blood agar slant and Sabouraud's agar, the culture underwent characteristic gross and microscopic changes with complete suppression of mycelium formation and development of only large budding cells. The progression of changes in the microscopic appearance of the culture is illustrated in Figures 7, 8, and 9. The third serial transfer of the organism produced a mealy, wrinkled, cerebriform and friable growth of fungus which possessed no cottony, aerial mycelium. It was noted that frequent transfers favored the mealy type of growth.

Growth Characteristics at Room Temperature. A single transfer of the organism growing at 37° C. to either Sabouraud's or blood agar at room temperature caused the prompt reversion of structure, with development of an abundant wooly, white, aerial mycelium (Fig. 6). Further study of the organism growing in a Henrici-type slide culture⁸ at room temperature revealed the development of abundant aerial mycelium and numerous oval to round conidia, 3 to 4 μ in diameter, which were attached to hyphae near the segmentations. Other round to pear-shaped conidia of the same size developed on lateral sterigmata of varying lengths (Fig. 10). Submerged mycelium developed raquette hyphae and thick, swollen structures resembling intercalary chlamydo-spores, 7.5 to 18 μ in diameter.

Growth Characteristics on Carrot Slant. At 37° C., the organism produced well isolated, warty, wrinkled, cerebriform colonies similar to those produced on blood agar, but considerably more raised. Ascospore formation was not observed at any time. The new strain of fungus appeared to be identical in all its characteristics with a stock laboratory strain of *Blastomyces dermatitidis*.*

Cultural Variation at 37° C. After several subcultures on blood agar and prolonged incubation at 37° C., minute prickly elevations consisting of closely packed filaments of true mycelium appeared on the surface of the growth. These were considered to be abortive coremia representing an attempt of the fungus to revert to the myceliated state. Both the stock and newly isolated strains of *B. dermatitidis* exhibited this characteristic, and also showed the other two cultural variations described for the fungus by Henrici.⁸ These forms were: (1) mealy colony, unicellular form at 37° C. (Figs. 5, 7, 8, and 9); (2) prickly,

* Furnished by Dr. Norman F. Conant, Duke University, Durham, N.C.

In support of these statements, we might point to the wide variety of fungi, as listed in Table II, which appear in exudates as oval, spherical, or oblong, refractile, fungal cells more or less resembling yeasts and capable of causing granulomatous lesions in tissues. In Table II there are also presented careful measurements of fungal cells and spores made at different ages of culture by one of us (M.L.L.) and compared with sizes reported in the literature. In this study, measurements were made of only one representative culture. Strain variation was not investigated. In one study of strain variation of *Microsporum* by Conant,¹⁰ spore dimensions were found to be too variable for species differentiation. The wide variation in dimensions between the smallest and largest spore in a given culture of fungus and between strains of the same species is presumably dependent upon many factors of growth and development, none of which were studied here. Differences in spore size among the several genera of fungi presented in Table II are sufficient, however, to permit rough identification of the fungi in human exudates and stained tissues. Cultures should be attempted in every case in which a fungus disease is suspected.

Technic of Primary Isolation

Media suitable for *B. dermatitidis*, as well as for other fungi, are Sabouraud's dextrose or maltose agar incubated at room temperature, and blood agar incubated at 37° C. Specimens are streaked by the gridiron technic on both media in such a manner as to yield isolated fungal colonies. Growth of pathogenic fungi on primary isolation will appear in most cases within 7 to 10 days on Sabouraud's dextrose agar at room temperature, but may sometimes require as long as 16 days. Many air-borne nonpathogenic fungi, which are laboratory contaminants, on the other hand, produce abundant, rapidly spreading aerial mycelium in as short a time as 48 to 72 hours and may overgrow the entire plate. Care must be taken to minimize contamination by air-borne fungal spores during the pouring, storage, and inoculation of the media.* The use of a small, closed room, rendered relatively dust-free and kept shut, will increase the proportion of successful primary isolations. Once a pure culture of a fungus is obtained, it is easier to maintain its purity on Sabouraud's agar slant than on an agar plate because of the reduced exposure to air-borne fungal spores. As soon as visible, filamentous, mold-like colonies develop in the agar along

* A new agar medium for primary isolation of fungi, obviating many of the difficulties due to air-borne contamination and inhibiting bacteria, recently has been developed by the senior author and is now undergoing clinical trial.

An account of this medium has been published since this manuscript was submitted. Littman, M. L., A culture medium for the primary isolation of fungi. *Science*, 1947, 106, 109-111.

the lines of streaking, one entire small colony is dug out with a platinum loop and transferred to a Sabouraud's agar slant. Although the young colony will usually not have developed spores, the vegetative mycelium is nevertheless quite capable of further growth and subsequent sporulation in the transplant.

All fungi incubating at room temperature are kept in a darkened cabinet since direct sunlight may inhibit growth. Streaking of a blood agar plate with fresh exudate and incubation at 37° C., as recommended by Martin and Smith,⁷ and Conant *et al.*,² will increase the likelihood of successful primary isolation. When the fungus is obtained in the pure state, free of bacteria and other fungi, attempts at identification may be continued by the slide culture technic, giant colony study, identification of characteristic sporulating elements in special media, and studies of animal pathogenicity and tissue reactions. If suitable facilities are not available locally, a pure culture may be sent to a specialized laboratory for identification.

Primary isolation and subsequent identification of pathogenic fungi from lesions in patients usually is not a difficult task for an experienced mycologist. It does present real difficulties to the bacteriologist or pathologist who is unfamiliar with the special methods and media required. Demonstration and primary isolation of certain fungi from mycotic lesions are simple if certain basic procedures are followed in the treatment of the exudate:

Direct Examination. (1) Mount in 10 per cent NaOH; (2) Gram stain or Wright stain; (3) incubation of unstained saline mount to detect sprouting or budding; (4) rough identification of the fungus with the help of Table II (spherical or oval fungal cells only).

Culture. (1) Streaking on Sabouraud's and blood agar plates, and incubation at room temperature and 37° C., respectively; (2) early transfer of young mold colony to Sabouraud's agar slant; (3) slide culture; (4) giant colony study; (5) sporulating media; (6) animal pathogenicity; (7) animal tissue reactions.

SUMMARY AND CONCLUSIONS

A case of fatal North American blastomycosis presented, on autopsy, involvement of the skin, subcutaneous tissues, lungs, liver, spleen, kidneys, prostate, bone, meninges, superior sagittal sinus, and brain. Histopathologic examination showed widespread abscesses and granulomatous foci in which the polymorphonuclear neutrophil was the predominating inflammatory cell.

Cultures isolated from exudates and from organs obtained at autopsy were identified as *Blastomyces dermatitidis*. Budding cell forms of the

Cryptococcosis	Oval to spherical organisms surrounded by irregular, nonstaining, mucinous capsule; single buds; best seen in Gram-stained sections.	Oval	5	Room	SAB	2.5 x 2.5	6.6 x 8.2	Culture# Exudate Exudate Exudate Tissue Tissue	4-5 5-20 5-10 2-15 5-15 5-10	6 2 14 8 6 14
Cryptococcosis <i>Cryptococcus neoformans</i> †	Oval to spherical, forming single buds, thick-walled, yeast-like organisms; wide refractile capsule demonstrable in India ink preparation.	Oval	35	Room	SAB	2.5 x 2.5	6.6 x 8.2	Culture# Exudate Exudate Exudate Tissue Tissue	4-5 5-20 5-10 2-15 5-15 5-10	6 2 14 8 6 14
Moniliasis <i>Candida albicans</i> †	Small, oval, thin-walled, yeast-like organisms, forming single buds.	Oval	5	Room	SAB	3.3 x 3.3	8.2 x 8.2	Culture# Exudate Tissue	3-6 2.5-4 4	18 2 2
		Oval	40	Room	SAB	2.5 x 2.5	9.0 x 9.0	Culture# Exudate Tissue	3-6 2.5-4 4	18 2 2
Coccidioidomycosis <i>Coccidioides immitis</i> †	Nonbudding, thick-walled spherules filled with numerous endospores which are released in tissues and which increase in size with maturity. Immature spherules not containing endospores are doubly contoured and resemble budding forms of <i>B. dermatitidis</i> and <i>P. brasiliensis</i> .	Spherule†			Tissue section	12.8 x 16.4	46.0 x 46.0	Exudate Exudate Exudate Tissue Tissue Tissue Tissue	2-80 20-80 5-50 2-80 2-60 20-80 6-50	14 2 8 14 15 8 6
		Endospore†			Tissue section	2.5 x 2.5	5.7 x 5.7	Exudate Tissue	2-5 1-4	2 8

TABLE II (Continued)

Disease and organism	In exudates ^{a,b,11}	In stained tissue section ^{a,b,11}	Measured size in culture and tissue section						Literature		
			Cell type or shape	Age of incubation days	Temperature	Medium	Width x length		Source	Size	Reference no.
Chromoblastomycosis <i>Hormodendrum compactum</i> [†] <i>Hormodendrum pedrosoi</i> [†] <i>Phialophora verrucosa</i> [‡]	All three forms are single or clustered, round, thick-walled, dark brown bodies; multiply by splitting and not by budding.	All three forms identical; brown, round, clustered, septate cells.					Smallest size	Largest size			
			Oval conidia	14	Room	SAB	1.6 x 1.6	2.0 x 2.0	Culture [#]	1.5-3.0	16
			Oval conidia [§]	14	Room	SAB	1.6 x 1.6	1.6 x 2.5	Culture [#] Culture [#]	1.5-2.5 [§] 1.5-6.0 [§]	16 2
			Oval conidia	14	Room	SAB	1.6 x 1.6	2.5 x 4.6	Culture [#]	1.5-4.0	16
Rhinosporidiosis <i>Rhinosporidium seberi</i>	Round to oval spores possessing thin cell walls and containing several granules of uniform size. Spores mature to large sporangia.	Numerous large sporangia which are empty or filled with many oval, thin-walled spores; the latter may also be found free in tissues.	Sporangia			Tissue section	21 x 21	177 x 206	Tissue	50-300	17
			Spores			Tissue section	3 x 3-8	12 x 12	Tissue	8-9	17

* Isolated from a case of fatal systemic blastomycosis.

† Furnished by Dr. N. F. Conant, Duke University, Durham, N.C.

‡ One tissue section.

§ *Phialophora* type of conidia.

|| Nasal mucosa, one case.

|| Sabouraud's agar.

Age of culture not reported.

△ Hormodendrum type of conidia.

coverslip on the preparation and gently warming the slide. Since numerous artifacts are easily confused with round or oblong fungal cells, a vaseline-rimmed mount of the fresh untreated specimen may be kept at room temperature for 24 to 48 hours and examined for either budding cells or development of hyphal germ tubes and hyphal elements from the suspected fungal spores.

Routine Gram staining of smears for demonstration of fungal elements is unsatisfactory because of the distorting effect of heat fixation. Gram stains of alcohol-fixed smears or direct Wright stains generally yield better results. Direct examination of unstained exudates in our case of blastomycosis disclosed the presence of numerous nonbudding, oval, thick-walled, refractile bodies, which, when maintained in a moist, untreated mount at room temperature for 24 hours, formed single, small buds compatible in structural characteristics with cells of *B. dermatitidis*. The wall of the oval cell was thick, highly refractile, and appeared as a bright band bordered by two thin, dark lines, which imparted a doubly contoured appearance to the unstained cell. The cell wall of the single bud, on the other hand, was considerably thinner than that of the parent cell.

Although the finding of budding, thick-walled, doubly contoured cells is suggestive of *Blastomyces*, it should be stressed that the doubly contoured appearance is by no means confined to this genus. Budding cells of certain species of *Torula* and *Candida* possess double contours which may be even more prominent than those of *Blastomyces*. Immature spherules of *Coccidioides immitis*, not possessing endospores, appear doubly contoured and resemble nonbudding forms of *Blastomyces dermatitidis* and *Paracoccidioides (Blastomyces) brasiliensis*. Furthermore, *Cryptococcus* cells possess rather thick cell walls and present double contours, as well as the spherical or oblong cells of *Geotrichum*. The discovery of budding, thick-walled, doubly contoured cells in exudates, although highly suggestive of *Blastomyces dermatitidis*, should, therefore, not be considered as ultimate proof of diagnosis of North American blastomycosis. As aptly phrased by Martin and Smith: ¹

"The diagnosis can be proved unequivocally only by the isolation and identification of the infecting organism, *Blastomyces dermatitidis*. However, from the history and clinical appearance of the infection, direct examination of material from the lesions, skin tests, and complement fixation reactions, tentative diagnoses can be made which should stimulate efforts toward isolation of the fungus."

Baker ³ believed that the tissue reaction produced by *B. dermatitidis* is sufficiently distinctive to permit histopathologic diagnosis with a high degree of accuracy if *Blastomyces*-like organisms are present in the lesion, but attempts should be made to obtain pure cultures in every case.

abortive coremia at 37° C.; and (3) wooly, mycelial form at room temperature (Figs. 6 and 10). In summary, it may be said that the morphologic appearance of *B. dermatitidis* on agar medium is variable, depending on temperature of incubation, age of the culture, and number and rapidity of transfers from the original isolation. Recent studies by Levine and Ordal⁹ on factors influencing the structure of *B. dermatitidis* on peptone glucose medium showed that the wooly, mycelial form was predominant in cultures growing from "room temperature" through 33° C., and the mealy, yeast-like form from 35° C. through 37° C.

Biochemical Reactions. Both the newly isolated strain of *B. dermatitidis* and the stock laboratory strain, which had been maintained in the budding cell stage at 37° C., were inoculated into beef infusion broth, pH 6.8, containing 1 per cent of one of the following sugars: maltose, lactose, sucrose, and dextrose. At 37° C., both strains developed abundant, colorless, submerged mycelium in the liquid media rather than the fragmented, mealy type of growth of the organism on solid media at 37° C. Cultures were maintained for 6 weeks, during which time growth of the fungus filled the media, but failed to produce any discernible gas or change in reaction. This does not preclude, however, the utilization of any of the carbohydrates by either strain of Blastomyces.

IN VITRO RESISTANCE OF BLASTOMYCES DERMATITIDIS TO STREPTOMYCIN AND PENICILLIN

Little information is available concerning the effect of the antibiotics, streptomycin and penicillin, on *B. dermatitidis*. Since the accepted method of treatment of North American blastomycosis with iodides and x-ray therapy is not particularly effective, the *in vitro* action of streptomycin and penicillin against the organism was thought worthy of examination.

Series of tubes of Sabouraud's dextrose broth and nutrient broth containing quantities of streptomycin hydrochloride (Merck) and sodium penicillin (Wyeth) varying from 0.1 to 200 units per cc. were inoculated with standard laboratory strains of *Staphylococcus aureus* and *Escherichia coli* as control organisms, and with two strains of *Blastomyces dermatitidis*, the newly isolated and the laboratory stock culture. The fungus inoculum consisted of 6-day-old budding cells, maintained in the unicellular state on blood agar at 37° C. Incubation was at 37° C. and observations were made at suitable intervals. The two strains of the fungus were found resistant to concentrations of streptomycin and penicillin up to 200 units per cc., as illustrated in

Table I. In order to verify these results, the "window" method of Cooke,¹⁰ originally devised for assay of penicillin and for the determination of penicillin sensitivity of bacteria, was used to determine the penicillin and streptomycin sensitivity of *B. dermatitidis*. Blood agar was used as the culture medium. Both strains of *Blastomyces* again were found to be insensitive to concentrations up to 200 units per cc.

TABLE I
In Vitro Resistance of *Blastomyces dermatitidis* to Streptomycin and Penicillin

Concentration	Growth in Sabouraud's dextrose broth			
Units per cc.	<i>Blastomyces dermatitidis</i> *	<i>Blastomyces dermatitidis</i> (Conant)	<i>Staphylococcus aureus</i> †	<i>Escherichia coli</i> †
Streptomycin hydrochloride				
0	+	+	+	+
0.1	+	+	+	+
0.5	+	+	+	+
1.0	+	+	+	+
5	+	+	+	—
10	+	+	±	—
20	+	+	—	—
50	+	+	—	—
100	+	+	—	—
200	+	+	—	—
Sodium penicillin				
0	+	+	+	
0.1	+	+	+	
0.5	+	+	—	
1.0	+	+	—	
5	+	+	—	
10	+	+	—	
20	+	+	—	
50	+	+	—	
100	+	+	—	
200	+	+	—	

* Isolated from case of fatal North American blastomycosis.

† Same results in nutrient broth.

of either antibiotic. The fact that penicillin did not affect the growth of two strains of *B. dermatitidis* when cultured at 37° C. on liquid or solid medium is in keeping with the clinical failure of penicillin therapy in a case of North American blastomycosis reported by Herrell, Nichols, and Heilman.¹¹ The results obtained by us are in agreement with the recent observations of Meyer and Ordal¹² who reported that streptomycin and penicillin were not fungistatic to one strain of *B. dermatitidis*.

DISCUSSION

Demonstration of Fungal Elements in Exudates

Pus and exudates are cleared by placing them in a drop of 10 to 20 per cent sodium hydroxide (or potassium hydroxide), mounting a

fungus were found to vary in size in culture from 4.6 by 5.7 μ (width and length) to 12.8 by 16.4 μ , and in various organs of the body from 4.6 by 6.6 μ to 12.3 by 14.0 μ .

Two strains of *B. dermatitidis* were found to be resistant to streptomycin and penicillin in concentrations up to 200 units per cc. For this reason it is not likely that either of these antibiotics will prove effective in the clinical treatment of North American blastomycosis.

Acknowledgments are made to Drs. Charles E. Dunlap, Guillermo M. Carrera, and Morris F. Shaffer of the Department of Pathology and Bacteriology, Dr. Roy H. Turner of the Department of Medicine, School of Medicine, Tulane University of Louisiana, and Dr. Emma S. Moss of the Department of Pathology, Charity Hospital, New Orleans, for their advice and guidance in the preparation of the manuscript.

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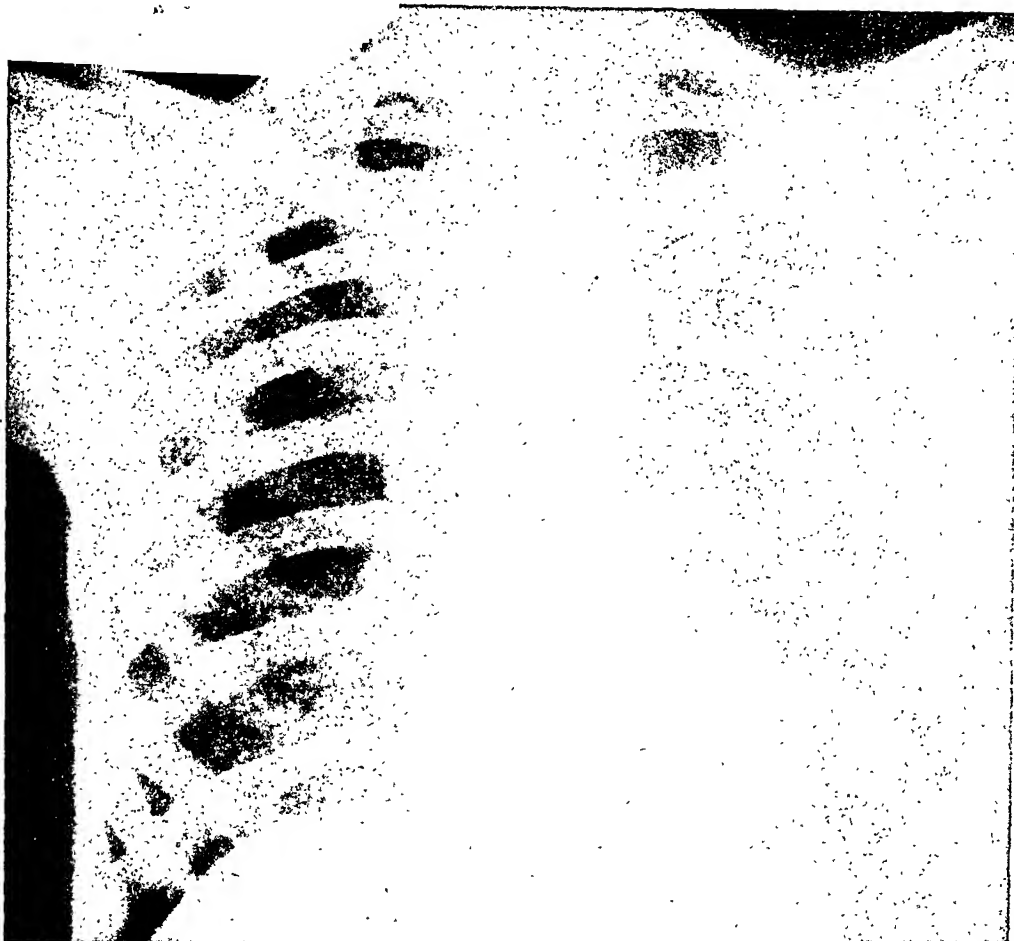
DESCRIPTION OF PLATES

PLATE 66

FIG. 1. Roentgenogram of the chest showing diffuse bilateral, miliary, pulmonary lesions in North American blastomycosis. Heart and mediastinal structures are within normal limits. The picture is compatible with miliary tuberculosis.

FIG. 2. Lung. The surface made by cutting shows firm, yellow nodules with no apparent central liquefaction.

1



2

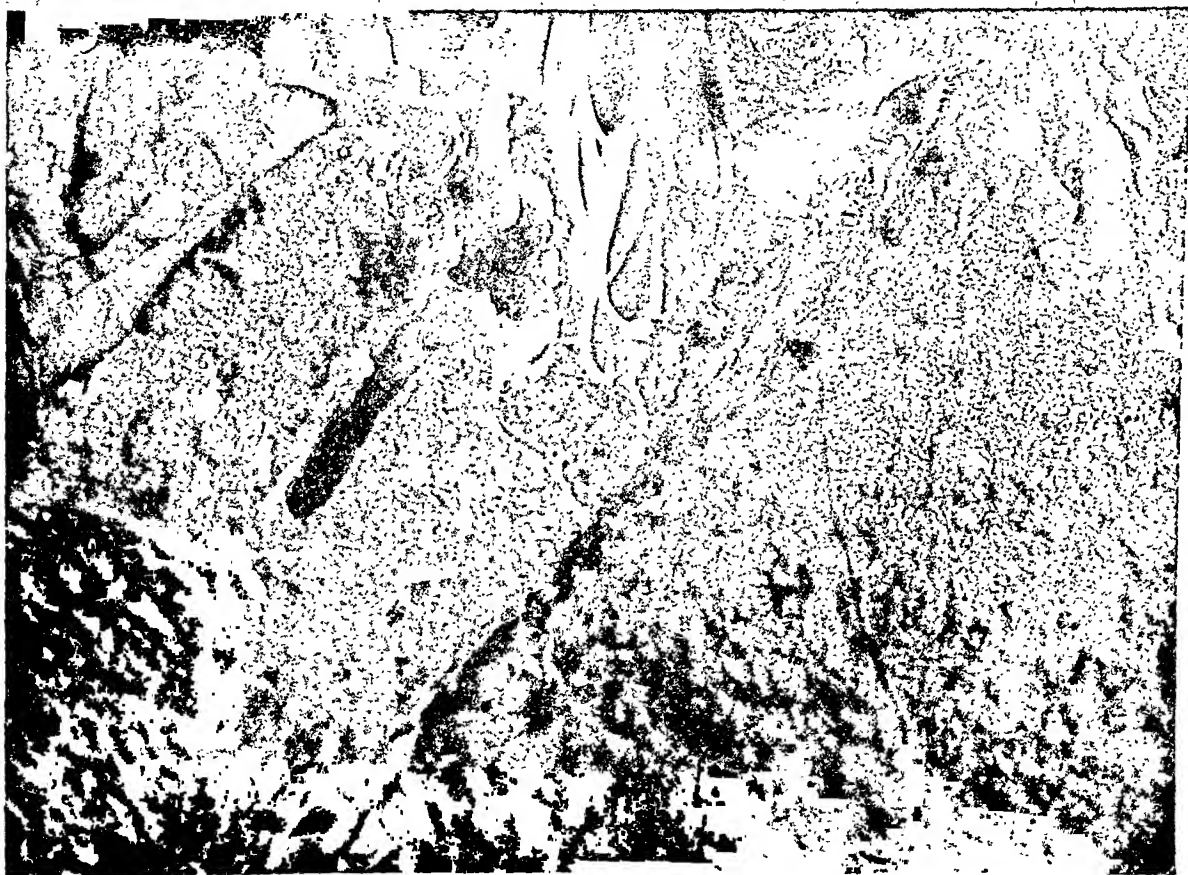
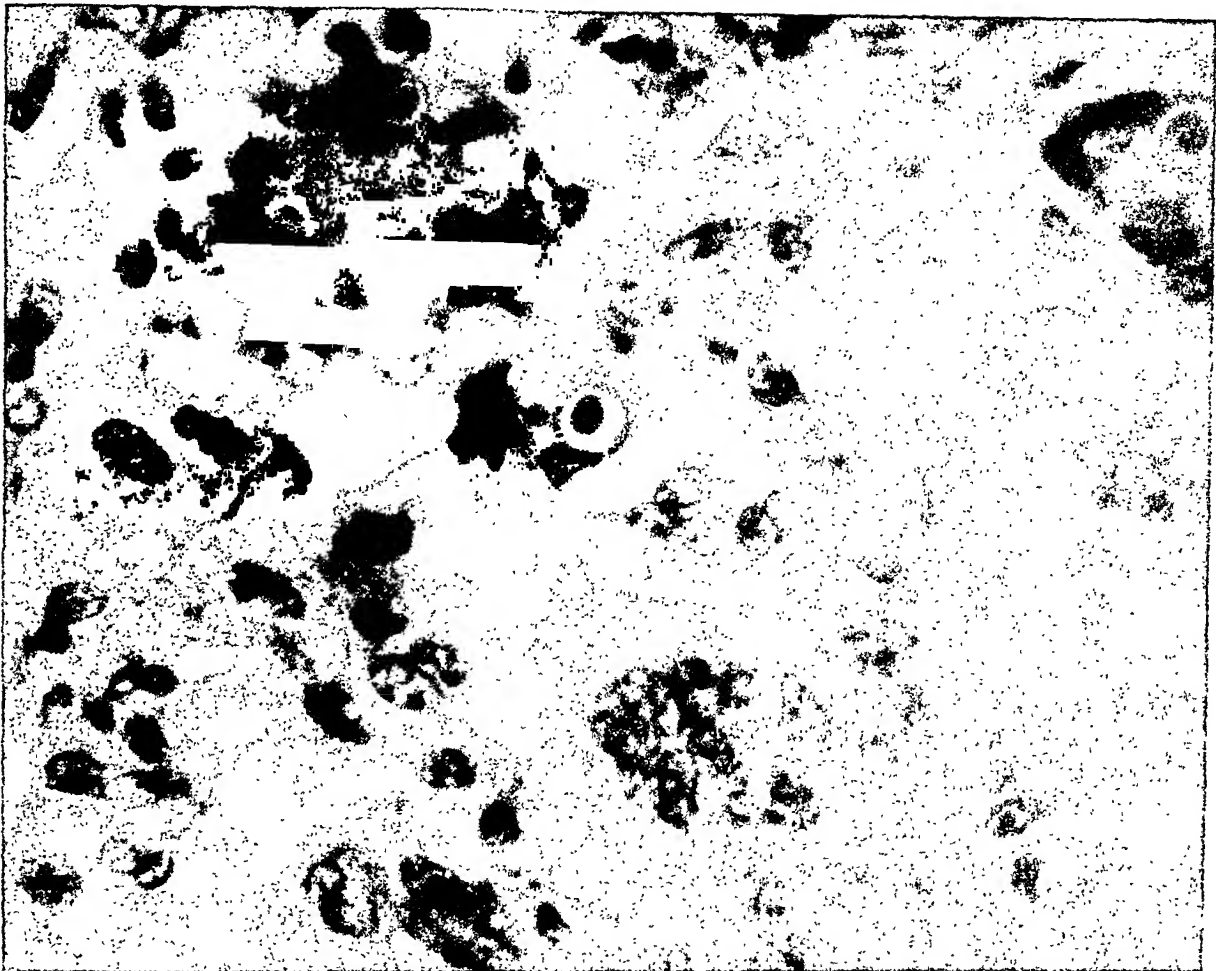


PLATE 67

FIG. 3. Lung. Photomicrograph of the periphery of a focal granuloma showing epithelioid cells and giant cells containing thick-walled, doubly contoured yeast-like bodies of *B. dermatitidis*. $\times 1000$.

FIG. 4. Brain. Superficial necrosis of the cortex of the parietal-occipital lobes near the midline. Of note are the thickening of the dura mater and the presence of a granulomatous exudate attached to the inner surface of the falx cerebri.

3



4

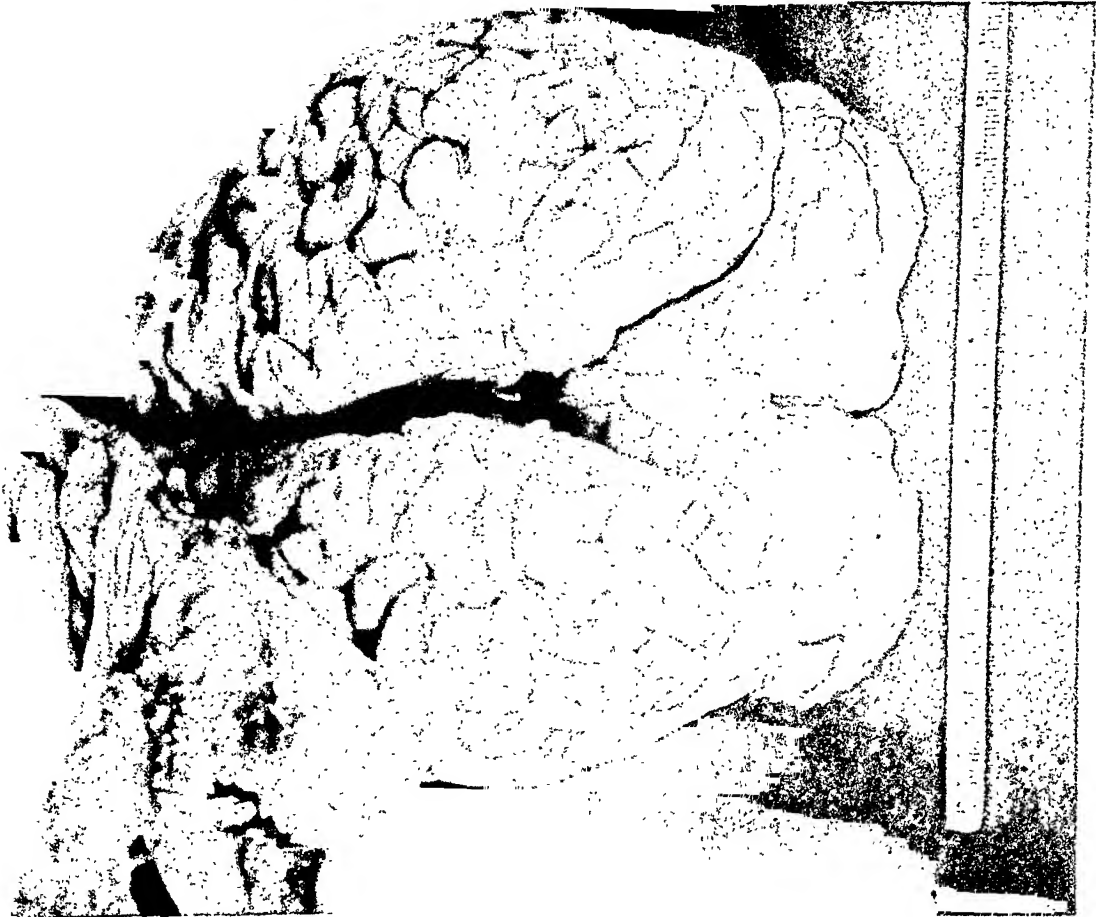


PLATE 68

FIG. 5. Giant colony of *B. dermatitidis*, incubated at 37° C. on Sabouraud's dextrose agar, showing typical mealy, wrinkled, cerebriform growth. The light gray periphery of the colony represents that portion of the growth which has penetrated into the agar. First transfer of newly isolated mold from growth at room temperature. (Mealy colony, original size.)

FIG. 6. Giant colony of *B. dermatitidis* incubated at room temperature on Sabouraud's dextrose agar, showing wooly mycelial appearance. (Wooly mycelial colony, original size.)

FIG. 7. Microscopic appearance of giant colony grown at 37° C., as seen in Figure 5. There are numerous round, doubly contoured, single or budding cells as well as numerous fragmented mycelial strands. First transfer from wooly growth at room temperature. (Mealy state.) $\times 1000$.

5



6



7

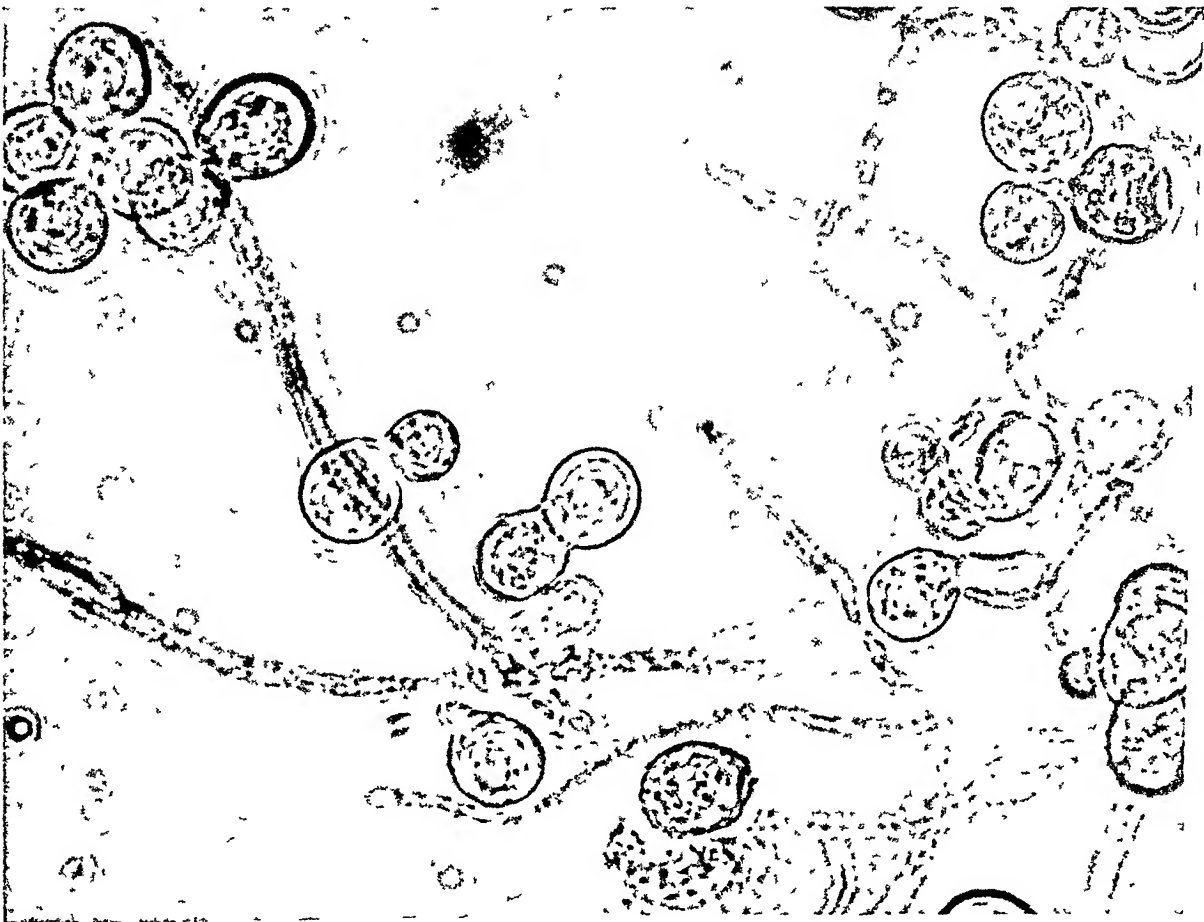
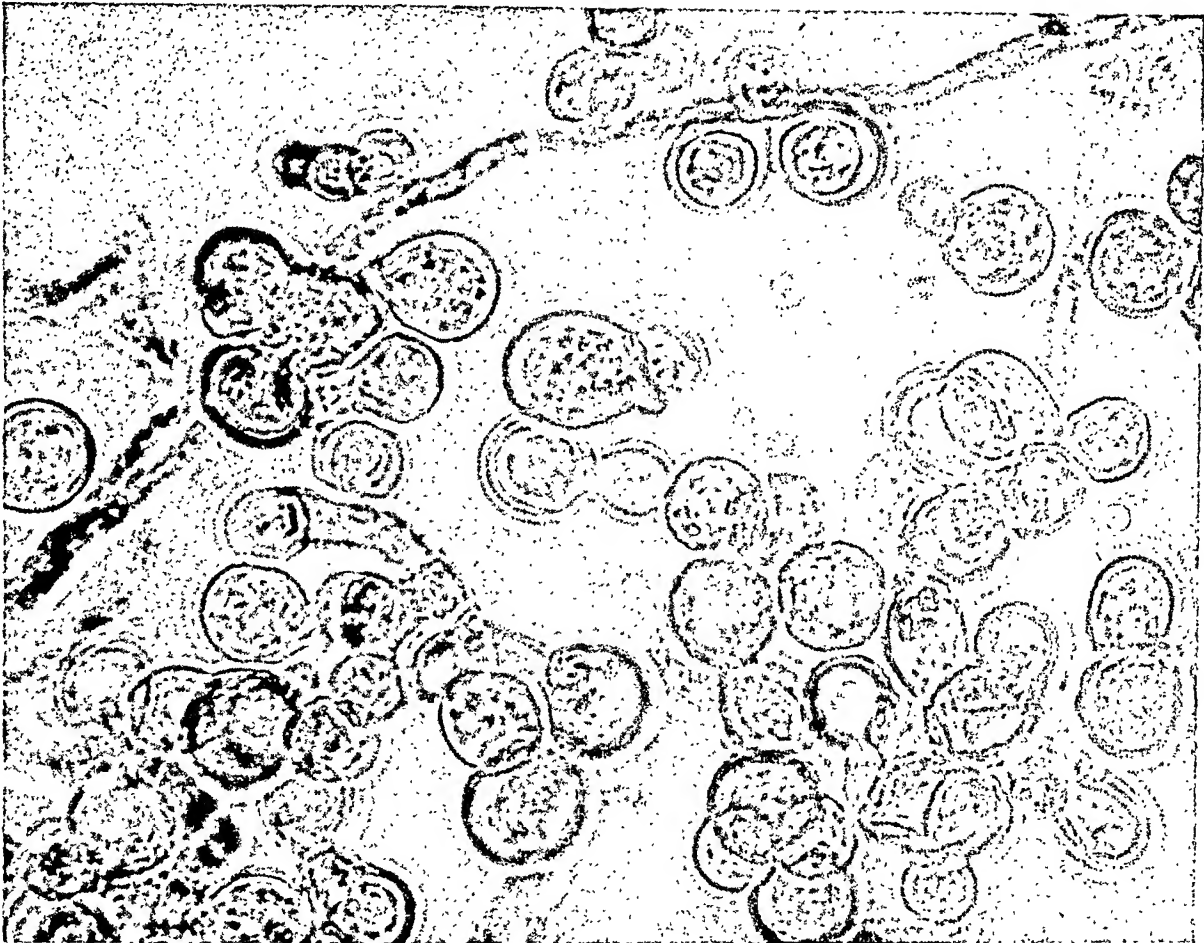


PLATE 69

FIG. 8. *B. dermatitidis*, second transfer, growth at 37° C. on Sabouraud's dextrose agar. There are numerous budding cells and relatively few mycelial strands. Age, 5 days. (Mealy state.) $\times 1000$.

FIG. 9. *B. dermatitidis*, third transfer, growing at 37° C. on Sabouraud's dextrose agar. There are numerous budding cells and very few mycelial strands. Age, 5 days. (Mealy state.) $\times 1000$.

88



89

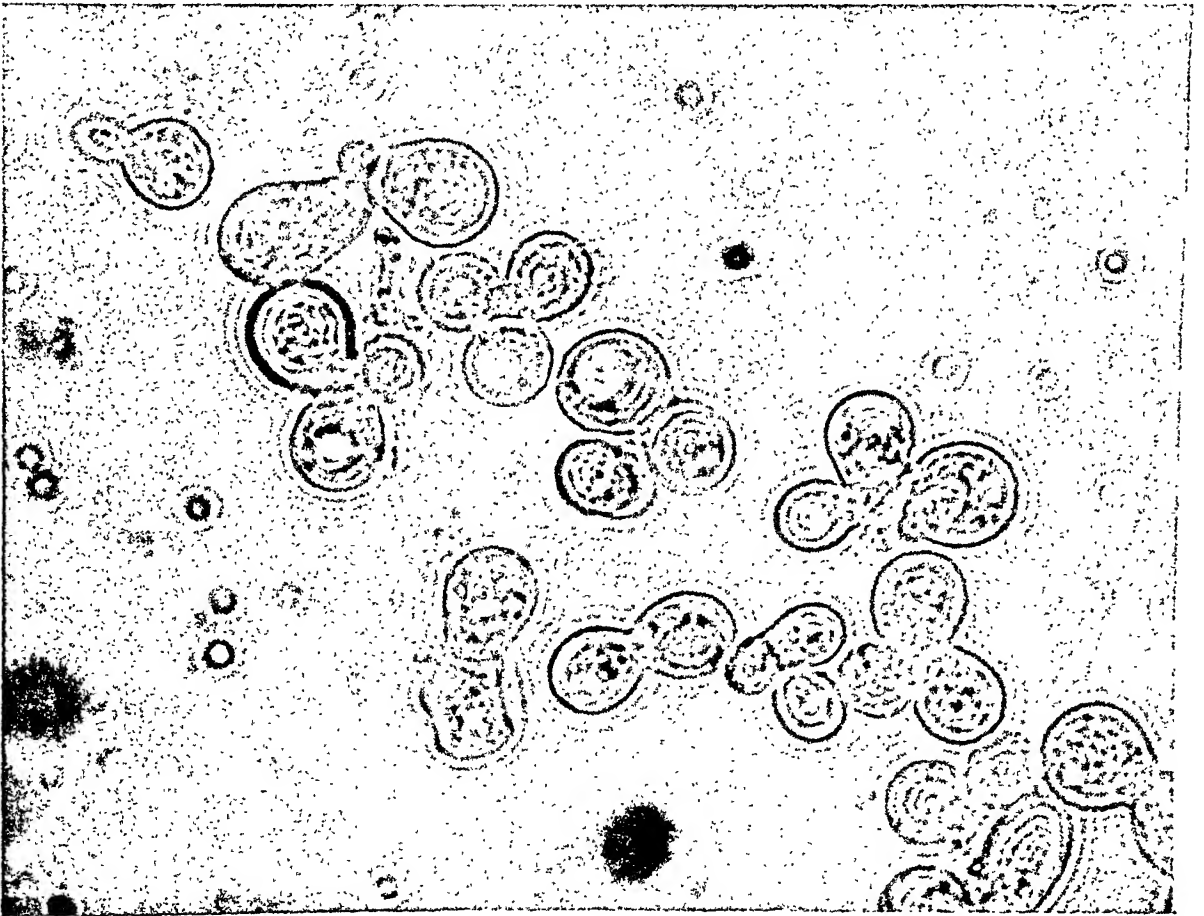


PLATE 70

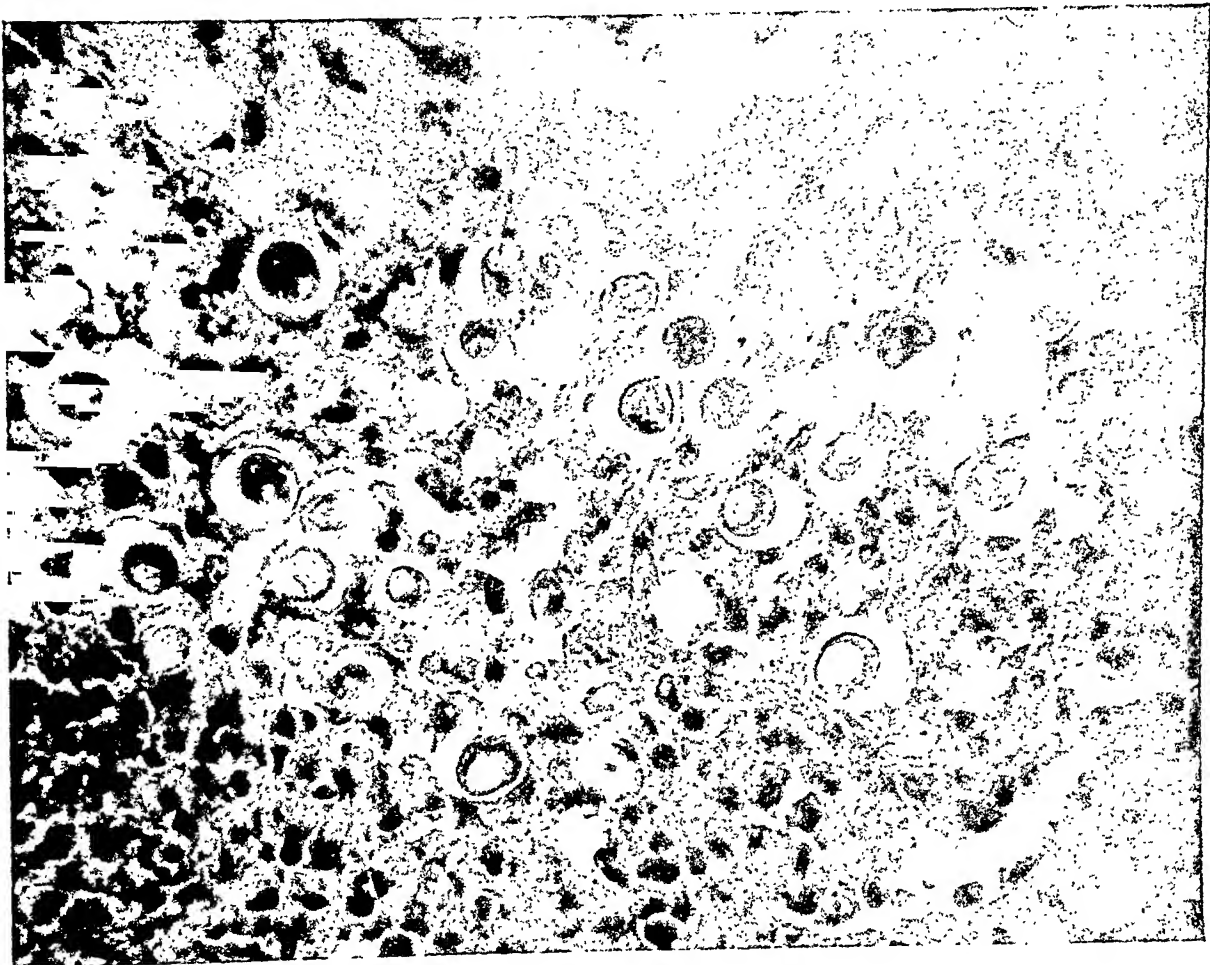
FIG. 10. Aerial mycelium of *B. dermatitidis* growing in a Henrici-type slide culture at room temperature. Oval, sessile conidia and other oval to pear-shaped conidia are attached to lateral sterigmata. Age, 14 days. (Wooly mycelial state.) $\times 1200$.

FIG. 11. Brain. Photomicrograph of involved portion of the right occipital lobe, showing numerous thick-walled budding cells, varying in diameter from 6.4 to 12.3 μ . Cytoplasmic staining of the fungal cells varies in intensity. $\times 1000$.

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PERSISTENT "INSECT BITES" (DERMAL EOSINOPHILIC GRANULOMAS) SIMULATING LYMPHOBLASTOMAS, HISTIOCYTOSIS, AND SQUAMOUS CELL CARCINOMAS*

ARTHUR C. ALLEN, M.D.†

(From the Army Institute of Pathology, Washington 25, D.C.)

In 1942, opportunity was afforded at the Army Institute of Pathology to review the histologic slides of a lesion said to have been produced by a tick bite. The microscopic sections seemed at the time indistinguishable from mycosis fungoides or Hodgkin's disease, especially in view of the presence of multiple lesions in the patient. However, following the study of the cutaneous reactions to arthropods (ticks, mosquitoes, and chiggers), it was quickly appreciated that not only were these diagnoses of neoplasia wrong but that the misinterpretation of these reactions was a common and serious error.¹ The errors involved the misconstruction not only of the dermal reaction but also of the epidermal changes. The latter response was confused with squamous cell carcinoma; the dermal infiltrate was mistaken for mycosis fungoides, Hodgkin's disease, lymphosarcoma, giant follicular lymphoblastoma, and Spiegler-Fendt sarcoid. Undoubtedly the principal reason for the failure to attribute these reactions properly to bites of arachnida and insects was referable to the general impression, despite clear-cut clinical histories, that such reactions last only for days, whereas, in truth, they may persist for as long as 2 years. More recently, the problem has been further complicated by introduction into the literature of a lesion called "eosinophilic granuloma of skin," an entity of questionable nosologic justification.²⁻⁴ Therefore, because of the major importance of establishing a definitive diagnosis and because of the interest in the pathogenesis of a much mimicked histologic pattern, it was felt of use to record the experience in this matter.

MATERIAL

There were available for study the histologic preparations of reactions to "bites" of 20 arthropods, including 9 ticks, 4 mosquitoes, 3 chiggers ‡ ("red bugs"), and 4 insects not otherwise identified. For comparison there were available the sections of 30 eschars of scrub typhus (tsutsugamushi disease) and 2 sections of skin infected with the ovum or larva of worms. The eschars produced by mites of the

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† Now at Memorial Hospital, New York 21, N.Y.

‡ The "chiggers" are the larval mites of *Trombicula irritans* and are to be distinguished from "jiggers," or adult sand fleas (*Pulex penetrans*).

species *Trombicula* offered no diagnostic problems inasmuch as they were all from active cases of scrub typhus. No examples of the late residue of such eschars were studied.

The lesions occurred on the extremities in 8 cases, on the abdomen in 5, on the back in 3, on the penis in 2, and in the axilla in 2. Their duration varied from 3 weeks to approximately 2 years; 6 were present longer than 1 year. All but one of the patients were white; in the one Negro, the lesion, following a tick bite, lasted 15 months before it was excised. Two of the group were females. The ages ranged from 21 to 49 years. In 3 instances, the ages were not recorded. Twelve of the 20 bites were acquired in California, Georgia, Texas, and Louisiana. The clinical appearance of the lesions varied from a patch of eczema such as might be caused by an irritant or dermatitis venenata, to a smooth, firm, erythematous, persistent, pruritic, slowly growing or stationary papule. The latter form of lesion simulated a fibroma. Some of the lesions were excoriated or ulcerated; some oozed, formed crusts, and desquamated. One of the lesions on the penis suggested a syphilitic chancre. There was a tendency for the ulcerations to heal, to recur subsequently, and to heal again. In such lesions a portion of the tick was left embedded in the skin. Marked pruritis was a particularly prominent and constant symptom. The lesions varied from 1 to 7.5 cm. in maximum diameter. It was generally impossible to estimate the duration from their clinical appearance.

FINDINGS

The histologic picture as stated had two distinct components which might or might not be combined: (1) changes in the epidermis and (2) the dermal infiltrate.

Epidermal Reaction

The most striking feature of the epidermal reaction was the marked pseudo-epitheliomatous hyperplasia which occurred in 7 of the 20 instances (2 chiggers, 2 ticks, and 3 unidentified). In 6 of the 7 cases, lesions with the epidermal hyperplasia were submitted to the Institute with the incorrect diagnosis of squamous cell carcinoma (Figs. 1 to 4). The pseudo-epitheliomatous hyperplasia was quite like that often seen at the margins of cutaneous ulcers. The differentiation of pseudo-epitheliomatous hyperplasia from carcinoma was by no means always a simple matter inasmuch as a disruption of the epidermal basement membrane and an excessive number of mitotic figures in the advanced portions of the rete pegs were occasionally present (Fig. 4). The diagnosis of hyperplasia rested on the conclusion that the isolated epi-

dermal nests were not truly invasive but were obliquely cut pegs actually continuous with the epidermis. Moreover, if the cells in mitosis were excepted, the other cells in these deep nests were essentially as differentiated as those of the neighboring uninvolved epidermis. The disruption of the basement membrane was regarded as the effect of the adjacent extensive, compact, inflammatory reaction. In this connection it was noted that pseudo-epitheliomatous hyperplasia was in each instance accompanied by marked dermal inflammation and was most pronounced over the densest portion of the dermal reaction.

Other changes in the epidermis included varying degrees of spongiosis (Fig. 6) which in 4 lesions (2 ticks, 1 chigger, 1 unidentified) reached the degree of vesiculation (Figs. 3 and 5). The vesicles were multiloculated, eczematous collections of serum containing purulent exudate or, as occurred in one instance, masses of eosinophilic leukocytes. The roof of the vesicles and the adjacent epidermis tended to be parakeratotic. Often loosely scattered neutrophilic and a few eosinophilic leukocytes were located in the epidermis, particularly in the spongiotic areas (Fig. 6). In the absence of pseudo-epitheliomatous hyperplasia a relatively uniform acanthosis, usually associated with hyperkeratosis and focal parakeratosis, was present. Occasionally the hyperkeratosis took the form of prominent keratinous plugs of follicles. The acanthosis was of a nonspecific variety but in some lesions simulated that found in neurodermatitis.

Dermal Infiltrate

The dermal inflammatory reaction was in most instances characterized by concentrated masses of cells involving the entire thickness of the dermis and part of the hypodermis, or distributed in dense patches in various portions of the dermis. The reaction showed no predilection for the cutaneous appendages but was often primarily collected about blood vessels. Whereas pseudo-epitheliomatous hyperplasia was accompanied by abundant dermal reaction, the reverse was not necessarily true. The important features of the infiltrate included: (1) Its density and usually great extent. (2) The presence of numerous mature eosinophilic leukocytes reaching a concentration of as many as ninety per high-power field. (3) The large numbers of plasma cells which varied greatly in size, many with two nuclei and rarely three, and some of them indistinguishable from the giant plasma cells of mycosis fungoides. (4) The abundance of histiocytes with cytoplasm which appeared to have undergone diffuse hydropic swelling and reacted negatively to stains for glycogen and fat. Mast cells were not increased. (5) Finally, there were noted conspicuous lymph follicles ,

(5 of the 20 cases) with definite germinal centers. It is of interest that eosinophilic leukocytes were excluded from the germinal centers despite their presence in large numbers in the adjacent infiltrate.

In addition, mitotic figures were observed in histiocytes in 5 of the lesions; karyorrhexis with phagocytosis of chromatin was common. Binucleate histiocytes with partially overlapping nuclei closely resembling the Sternberg-Reed cells of Hodgkin's disease also were found occasionally. In 3 lesions subepidermal edema, almost urticarial, was present; in only one of these was the process of short duration (Fig. 14). In 3 instances there were subendothelial edema, swelling of endothelial cells, and a few polymorphonuclear leukocytes in the intima of dermal arteries and veins, a reaction reminiscent of one form of allergic inflammation of vessels. In 4 other lesions (1 tick, 1 mosquito, and 2 unidentified) epidermal inclusion cysts were found in the mid-dermis in the core of the infiltrate. In one lesion portions of the tick actually were included in the center of an epidermal cyst (Figs. 11 and 12). In 2 cases there was noteworthy proliferation of the squamous cells lining these cysts. Foreign body giant cells were present in the vicinity of the cyst, possibly representing a reaction to the keratin of the epidermal inclusion or to broken off remnants of the arthropod. The apparent integrity of the cyst with no obvious extrusion of keratin and the association of eosinophilic leukocytes with the giant cells suggest that portions of the arthropod or its products might be the inciting agent.

Comparative Lesions

The acute lesions of the eschars of scrub typhus of approximately 9 to 27 days' duration differed strikingly from the reaction to other arthropods, essentially in the almost complete absence of eosinophilic leukocytes from the dermal infiltrate.⁵ In addition the constant superficial necrosis of the eschars was not noted in the lesions under current study. On the other hand the dermal reaction to the larva of *Ascaris* and to the ovum of *Schistosoma japonicum* was in basic respects similar to the bites of arthropods although the pseudo-epitheliomatous hyperplasia was lacking (Figs. 23 and 24).

DISCUSSION

Differentiation from Lymphoblastomas

The basic histologic picture of the cutaneous reaction to a variety of arthropods is a dense dermal infiltration consisting principally of mature eosinophilic leukocytes and plasma cells admixed with histiocytes which are occasionally in mitosis or binucleated, resembling Sternberg-Reed cells. This histologic reaction has been confused with

Hodgkin's disease and mycosis fungoides, a possibility noted previously by others.⁶ The occasional addition of circumscribed collections of lymphocytes, sometimes with large lymphoid follicles and actual germinal centers, may suggest giant follicular lymphoblastoma, Spiegler-Fendt sarcoid, or an ill defined atypical lymphoblastoma. The presence in histiocytes of mitotic figures, however rare, serves further to camouflage the true diagnosis inasmuch as mitotic figures in dermal infiltrates must be regarded as presumptive evidence of neoplasia until proved otherwise. The reaction to the bite of arthropods is a definite exception to this rule. Furthermore, contrary to the observations of others,⁶ these studies indicate that the reactions to the bites of arthropods may persist as active dermal "eosinophilic granulomas" for at least 2 years, and perhaps longer. It is precisely this fact, hitherto obscured, which requires that these lesions be considered in an altogether new stratum of differential diagnosis characterized by diseases of relatively long duration, particularly neoplastic processes. It bears noting that while the existence of a single lesion may to some degree support the diagnosis of insect bite as against neoplasm, this type of evidence must be tempered by the fact that lymphoblastomatous involvement of skin may appear as an isolated lesion for a long time. Conversely, insect bites may be multiple.

Hodgkin's disease may cause the most difficulty in differential diagnosis. The main basis for the histologic differentiation of the insect bites from the neoplastic lesions and Spiegler-Fendt sarcoid (whatever its nature) is the abundance of mature eosinophilic leukocytes in association with plasma cells. Evidence of phagocytosis is additional presumptive, but not conclusive, evidence against the diagnosis of lymphoblastoma. However, there are other criteria which lend further aid in differential diagnosis. Frequently the reaction to the arthropod is accompanied by a degree of pseudo-epitheliomatous hyperplasia of the epidermis that may be mistaken for squamous cell carcinoma. Such a reaction in conjunction with the suspected dermal infiltrate is, *per se*, potent evidence in favor of the reaction to an arthropod. Moreover, the presence of epidermal inclusion cysts, especially with foreign body giant cells intermingled with eosinophilic leukocytes, even in the absence of identifiable parts of the arthropod, is significant evidence of the inflammatory basis for the lesion.

Differentiation from Other Eosinophilic "Granulomas"

Perhaps the source of greatest confusion lies in the recently publicized group of "eosinophilic granulomas of the skin."^{3,4,7} There can be little question of the heterogeneity of this group in which there

appear to have been lumped lesions due to parasites of various sorts as well as to biting insects and acarines, lipoidal and reticulohistiocytoses including Letterer-Siwe's disease, periarteritis nodosa, and lymphoblastomas, particularly Hodgkin's disease. Surely, therefore, the tendency to consider the cutaneous "eosinophilic granuloma" an entity will serve only to add confusion. The differential histologic characteristics mentioned above, namely, eosinophilic plasmacellular infiltrate, epidermal inclusions, and pseudo-epitheliomatous hyperplasia, may aid the very practical purpose of segregating the reactions to the bites of arthropods from the other types of so-called eosinophilic granulomas. In the absence of pseudo-epitheliomatous hyperplasia, the reaction to the intracutaneous larvae of worms, often in the clinical form of "creeping eruption," may be indistinguishable from that of insect bites. The dermal eosinophilia in some cases of periarteritis nodosa may be fully as marked as in the lesions due to arthropods but the vascular change in the latter is relatively mild and does not present the fibrinoid degeneration that characterizes periarteritis nodosa, or, for that matter, erythema elevatum diutinum, another disease that has been included in the differential gamut. Various stains for fat that are clearly positive serve to rule out insect bites in favor of the lipoidal histiocytoses. However, the vacuolated cells of the former may be mistaken for lipoidal histiocytes in routine sections, although the granularity and fine vacuolization of the histiocytes is more evident in the histiocytoses, both lipoidal and nonlipoidal. The frequent presence of eosinophilic leukocytes in the histiocytoses calls for nice judgment in deciding that the histiocytes constitute the primary cellular response and, generally, are structurally different from those found in reactions to arthropods. Because of the identity of the names, the eosinophilic granuloma of skin has naturally been compared with the corresponding lesion of bone.⁷ Here again, in the lesion of the bones, the histiocytes with the acidophilic, finely granular, often lipoid-filled cytoplasm appear to be the essential, primary cellular matrix rather than the eosinophilic leukocytes, however abundant they may be. This is not meant categorically to deny any possible relationship between eosinophilic granuloma of bone and other forms of cutaneous eosinophilic granulomas of skin, exclusive, of course, of those due to arthropods. In the previously reported mixed group of "eosinophilic granuloma,"³ an eosinophilia of the peripheral blood commonly was present and was often at a high level. Data on the level of eosinophilic leukocytes in patients with arthropod bites included in the current study are incomplete, but in the 2 cases in which counts were made, no peripheral eosinophilia was present.

Pathogenesis

No constant or significant differences were noted in lesions varying in duration from 3 weeks to 2 years. Possibly a study of a larger series of cases in the early stages may modify this impression. In only one instance (mosquito bite) was a scar formed. The duration of this lesion was unknown. In any case, it is indisputably true that the prominence of eosinophilic leukocytes and plasma cells was as marked or even greater after 1 to 2 years as after 3 weeks. This constancy in the quality of the reactions notwithstanding their duration, which is at sharp variance with the observations of others,^{2,6} is of considerable interest because it implies that the stimulating agent of the arthropod or its venom in some way manages to maintain its activity over many months. This activity is achieved in the absence of any recognizable remnants of the arthropods, although in several instances there was a history of incomplete removal of a tick. Moreover, the duration of vesiculation observed in more than one-third of the cases appeared to be in days or, at most, weeks rather than in months. The pathogenesis of this acute or subacute vesiculation, occasionally recurrent in a chronic lesion, remains to be explained on a basis more adequate than mere excoriation. Inasmuch as these vesicles are of eczematous types, action of the persistent allergen within the lesions seems a likely basis for their development. There was no correlation between the occurrence of vesiculation and the duration of the lesions.

In contrast to the reactions of the skin to ticks, mosquitoes, and chiggers is the histologic picture of the eschar or primary lesion of scrub typhus (tsutsugamushi disease). In the eschar, eosinophilic leukocytes are absent or rare. This disparity is of interest because of the close taxonomic relationship of the larval mite of scrub typhus (*Trombicula akamushi* and other species) to the chigger (larval mite of *Trombicula irritans*). Of course, in the eschar there is the added factor that rickettsiae are included in the lesion, but these organisms are said not to affect the histologic reaction qualitatively.⁸

One of the lesions, of 15 months' duration produced by a tick, occurred in a Negro. Histologically, there was no appreciable epidermal reaction but considerable dermal infiltration. The reaction did not differ basically from those in the white patients, which is of interest in view of the known resistance of the skin of the Negro to reactions to lice, mites, and other arthropods.

Pseudo-epitheliomatous Hyperplasia

Finally, there remains the interpretation of the principal epidermal reaction or pseudo-epitheliomatous hyperplasia. The term pseudo-

epitheliomatous hyperplasia is used commonly in the dermatologic literature, but appears aptly chosen notwithstanding the resistance to it by general pathologists. The term does not connote a "precancerous" state. It refers simply to hyperplastic epidermis which sends shoots of branching rete pegs deep into the dermis so as to *simulate* a squamous cell carcinoma. This simulation becomes especially marked when islets of epidermis appear invasive; actually they belong to the tentacular epidermis which has been cut obliquely. This reaction, which occurs often at the margin of chronic cutaneous ulcers, was observed frequently in the reactions to arthropods and usually was mistaken for carcinoma. It is stated in the literature^{9,10} that the bites of insects have produced carcinomas. There is also a report of melanoma following a tick bite,¹¹ but this case appears to have had incomplete histologic study. However, the demonstration of a causal relationship in the bite of the arthropod to the development of the neoplasms, while of considerable fundamental interest and by no means disproved, still requires more direct evidence than is afforded by the available data.

SUMMARY

A histologic study was made of the reactions to the "bites" of ticks, chiggers, mosquitoes, and unidentified arthropods.

The reaction, which consisted of a dense dermal infiltrate characterized by large numbers of eosinophilic leukocytes, plasma cells, and histiocytes, may be mistaken for Hodgkin's disease, mycosis fungoides, atypical lymphoblastoma, histiocytoses, and the heterogeneous group of "eosinophilic granulomas."

The lesion is often associated with a pseudo-epitheliomatous hyperplasia which may be confused with squamous cell carcinoma. The association with an eosinophilic dermal infiltrate and with epidermal inclusion cysts provides helpful differential clues.

With one exception, no basic difference was noted in the histologic reaction of the skin to the various arthropods studied. The striking exception is the almost complete absence of eosinophilic leukocytes in the eschar or primary lesion of scrub typhus caused by the larval mite (*Trombicula akamushi* and related species).

It is emphasized that the reaction to the "bites" of arthropods may persist for many months and that, in general, no appreciable difference is noted in the histologic reaction in lesions lasting from 3 weeks to 2 years. It is therefore concluded that the stimulating agent of the arthropod somehow must persist actively in the focus of these lesions for a remarkably long time.

A single cutaneous lesion with the histologic picture suggestive of

Hodgkin's disease or other lymphoblastoma should always be suspected as having been caused by the bite of an arthropod until conclusively proved otherwise. The history of an insect bite may not be volunteered after a lapse of many months.

The cutaneous reactions of individuals, even of the same race, to different arthropods varies not only in the acute stage but also in the persistence, degree, and quality of the histologic reaction.

It remains to be determined precisely what agent in the venom or embedded parts of the arthropod, or both, is responsible for the cutaneous reaction.

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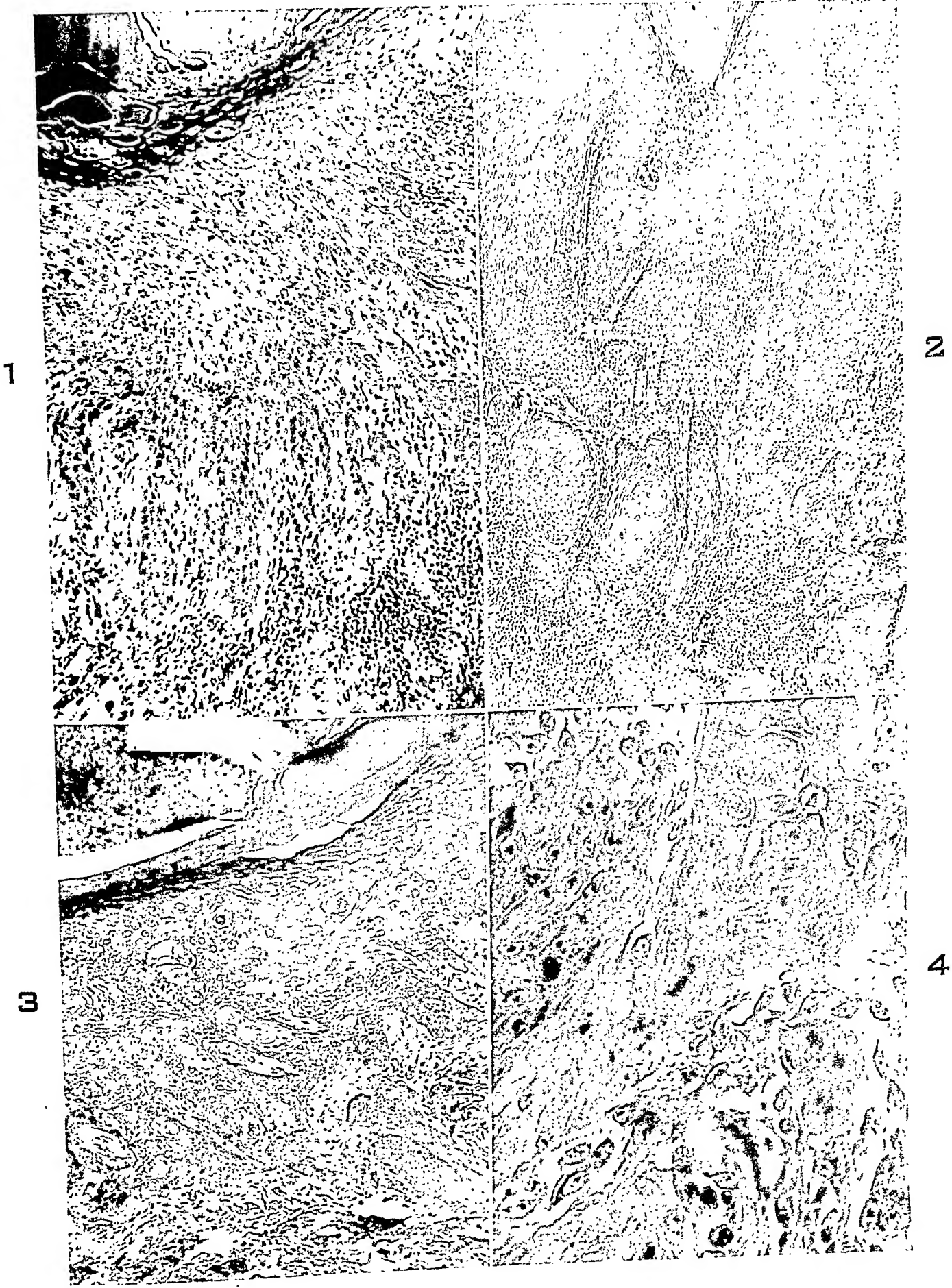
[Illustrations follow]

DESCRIPTION OF PLATES

All photomicrographs were made from sections stained with hematoxylin and eosin.

PLATE 71

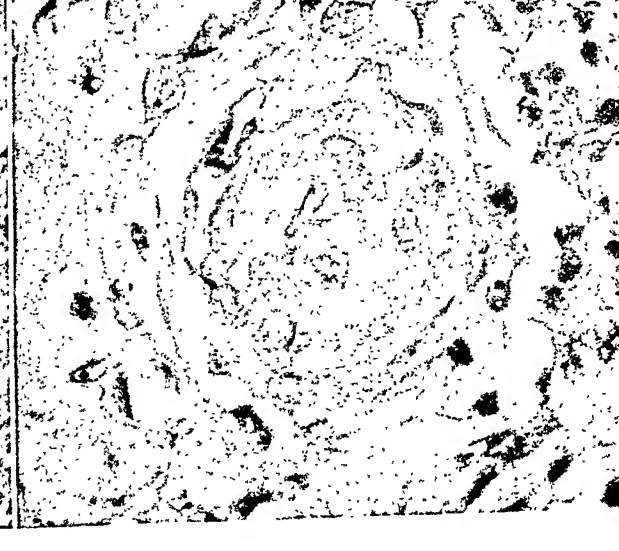
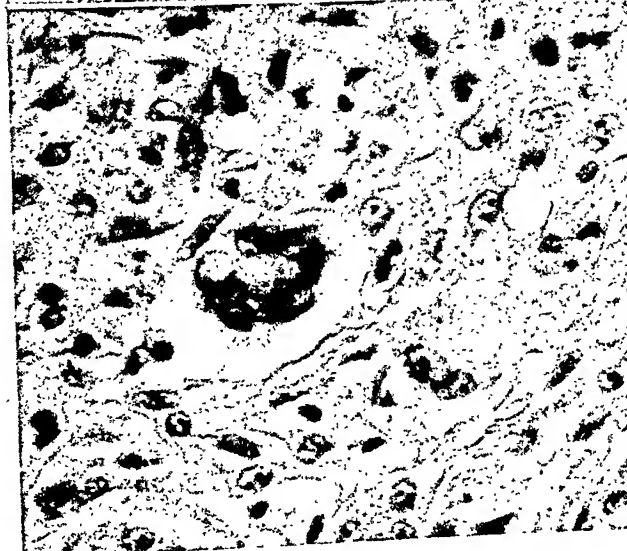
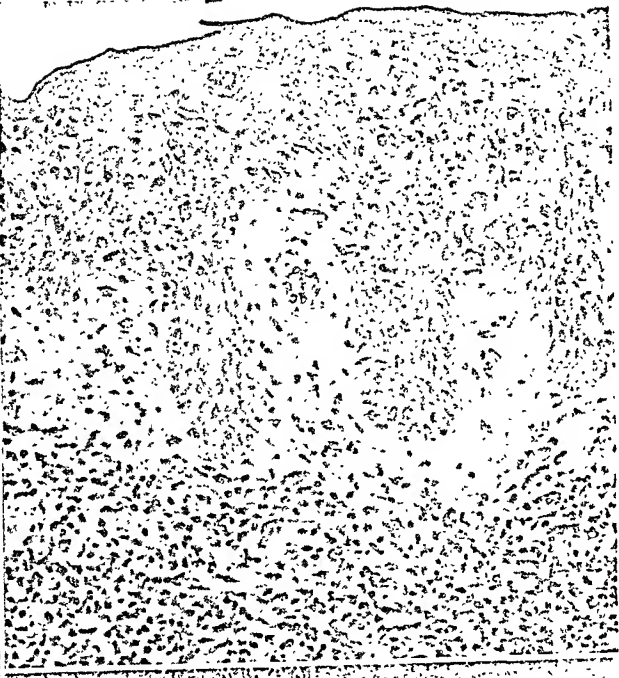
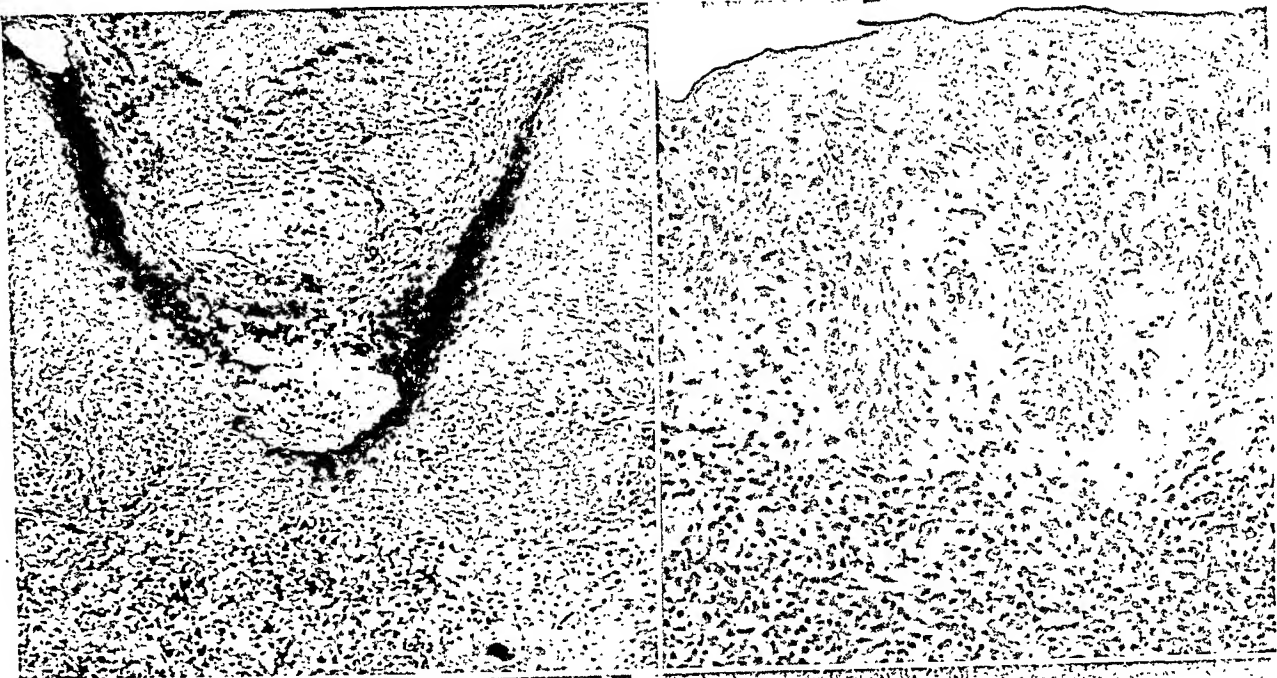
- FIG. 1. Pseudo-epitheliomatous hyperplasia $1\frac{1}{2}$ years after an insect bite. (Army Institute of Pathology negative no. 95762.)
- FIG. 2. Pseudo-epitheliomatous hyperplasia 4 months after a tick bite. (A.I.P. neg. 95767.)
- FIG. 3. Pseudo-epitheliomatous hyperplasia 8 months after a chigger bite. (A.I.P. neg. 99865.)
- FIG. 4. Higher magnification of the tips of the pegs of epidermis seen in Figure 3, showing numerous mitotic figures. (A.I.P. neg. 100469.)



Persistent "Insect Bites"

PLATE 72

- FIG. 5. Vesiculation, acanthosis, and dermal reaction 4 months after an insect bite. (A.I.P. neg. 99868.)
- FIG. 6. Spongiosis and epidermal transmigration of eosinophilic and neutrophilic leukocytes 4 months after a tick bite. The dermal infiltrate is conspicuous. (A.I.P. neg. 100435.)
- FIG. 7. Epidermal inclusion cyst 7 weeks following a mosquito bite. (A.I.P. neg. 99869.)
- FIG. 8. Giant cell proliferation together with plasma cells, eosinophilic and neutrophilic leukocytes comprise the reaction to the epidermal cyst seen in Figure 7. No parts of the insect were detectable. (A.I.P. neg. 100442.)
- FIG. 9. Giant cells admixed predominantly with eosinophilic leukocytes and histiocytes. This reaction was observed 7 weeks following a mosquito bite. (A.I.P. neg. 100471.)
- FIG. 10. Arteriolitis with swelling of cells and fibers of the entire wall, 4 months after an insect bite. (A.I.P. neg. 100467.)

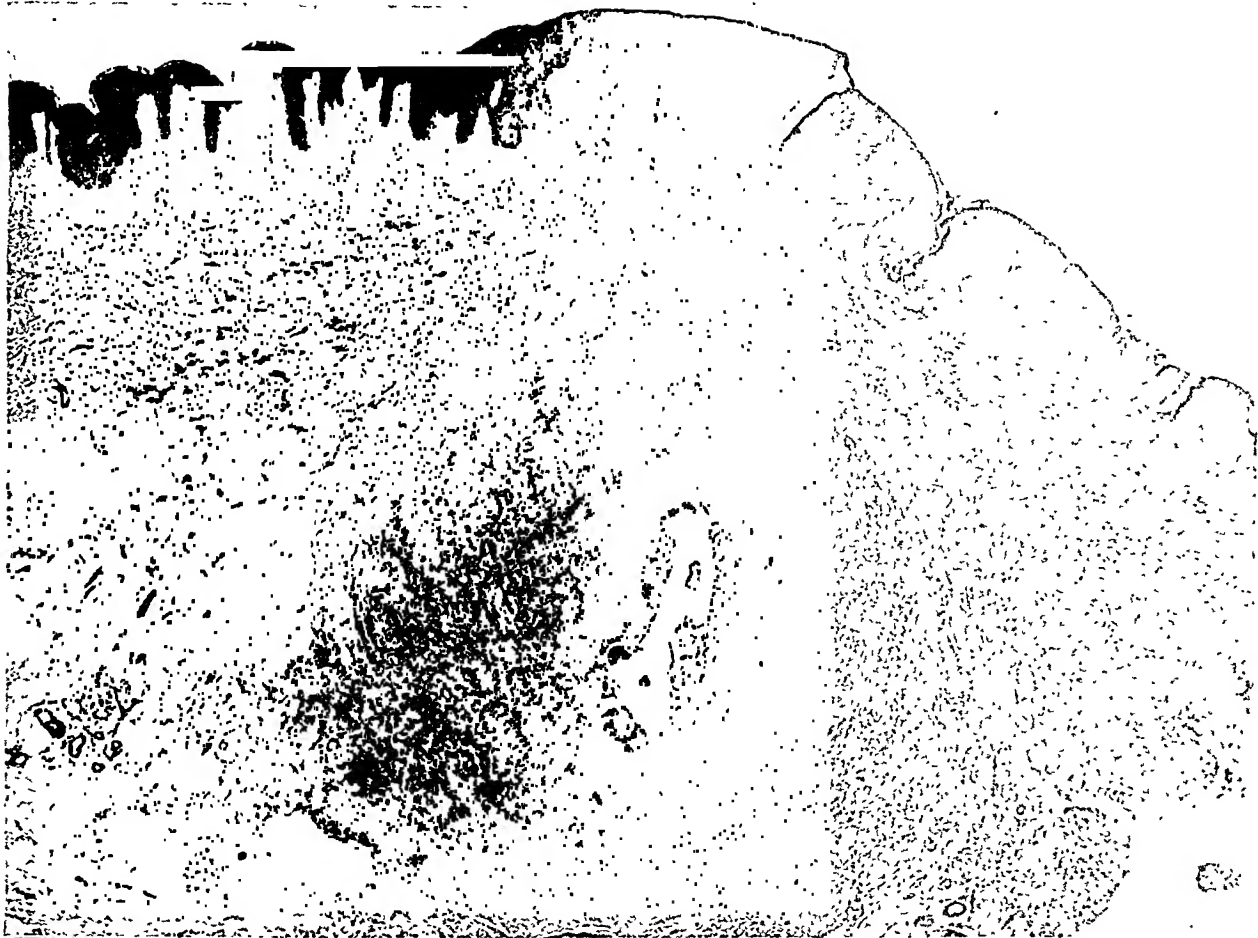


Persistent "Insect Bites"

PLATE 73

- FIG. 11. Reaction to a tick bite after $1\frac{1}{2}$ years, showing the dense dermal infiltrate ("eosinophilic granuloma") surrounding an epidermal inclusion cyst. The contents of the cyst include portions of the tick. (A.I.P. neg. 77486.)
- FIG. 12. Higher magnification of the epidermal inclusion cyst and the enclosed parts of the tick shown in Figure 11. (A.I.P. neg. 90949.)
- FIG. 13. Higher magnification of Figure 12, showing parts of the tick and epithelium of the inclusion cyst, several cells of which are in mitosis. (A.I.P. neg. 100470.)

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PLATE 74

FIG. 14. Dermal reaction 4 weeks after a tick bite, showing subepidermal edema and a dermal infiltrate in which the predominant cell is an eosinophilic leukocyte. (A.I.P. neg. 100613.)

FIG. 15. Dermal reaction ("eosinophilic granuloma") to a chigger bite after 10 months. (A.I.P. neg. 95765.)

FIG. 16. Dermal polymorphous reaction ("eosinophilic granuloma") to a tick bite after 18 months. (A.I.P. neg. 78276.)

FIG. 17. Reaction to a chigger bite after 8 months. The plasmacellular response simulates a syphilitic infiltrate. (A.I.P. neg. 80169.)

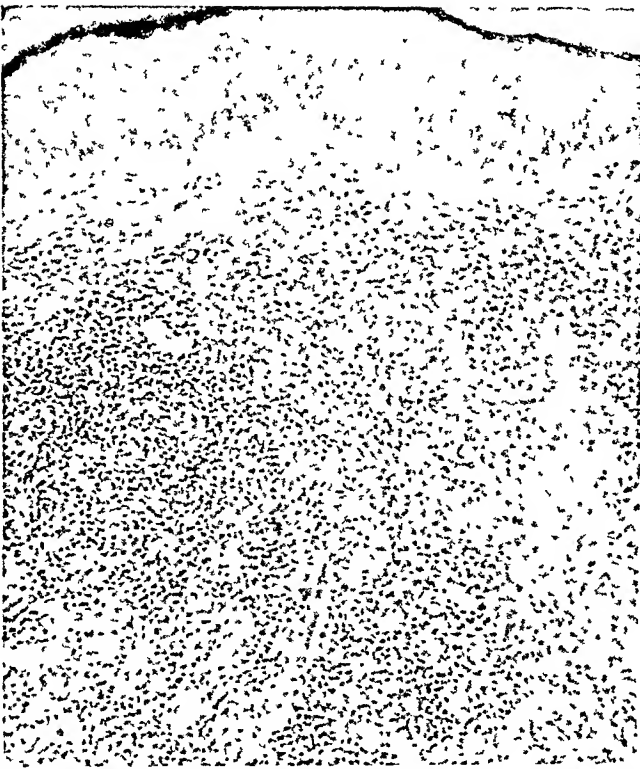
FIG. 18. Large binucleated cell, and numerous plasma cells and eosinophilic leukocytes, in the inflammatory response 8 months after a chigger bite. (A.I.P. neg. 100439.)

FIG. 19. A mitotic figure and many histiocytes with hydropic cytoplasmic changes in reaction 15 months after a tick bite. (A.I.P. neg. 100466.)

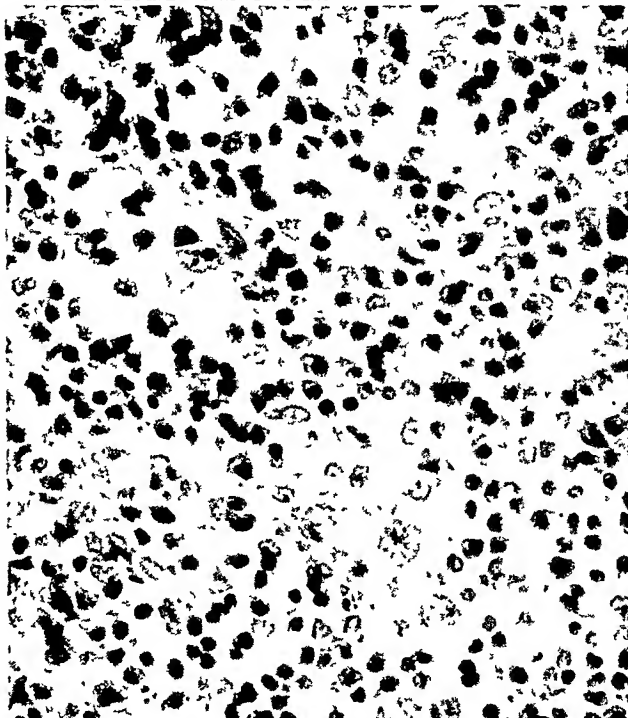
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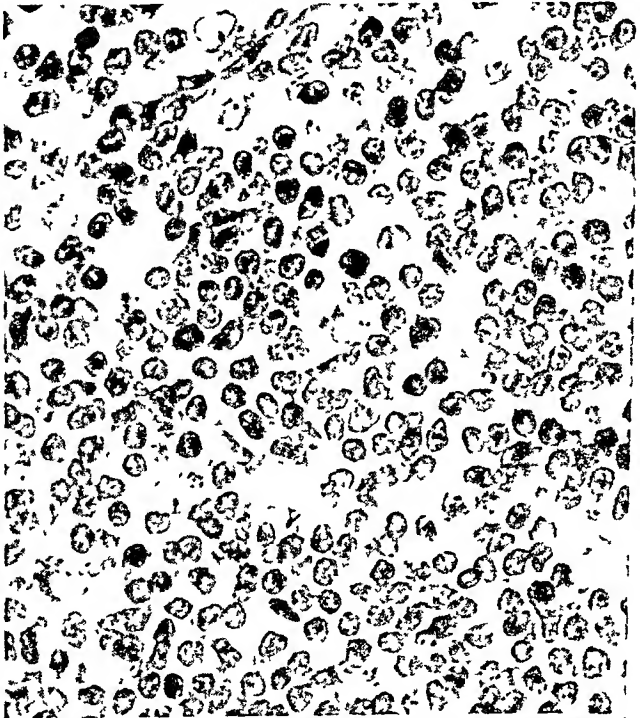
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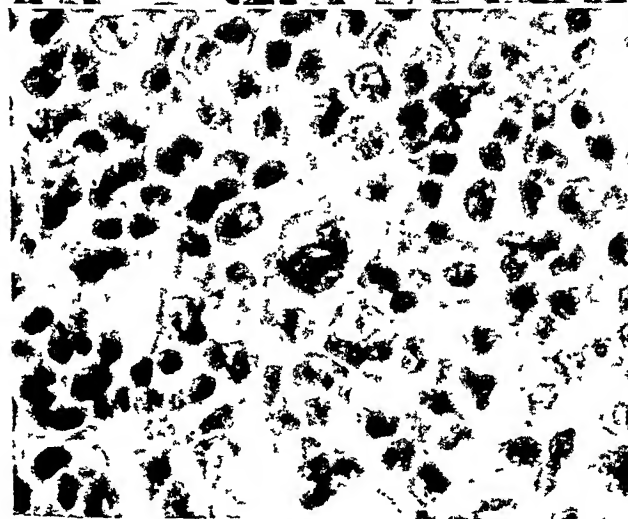
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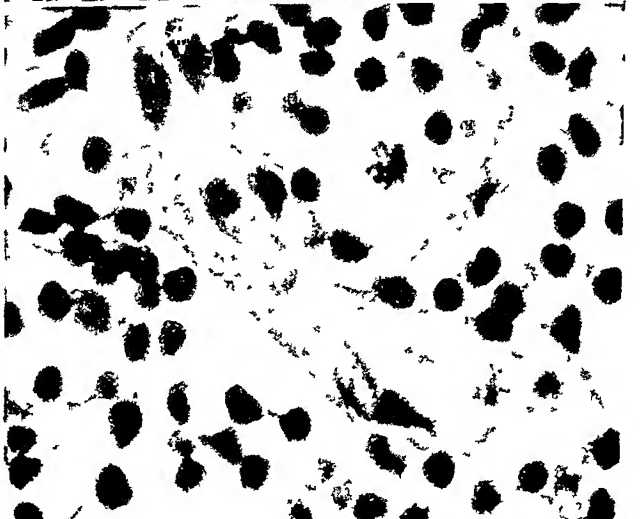


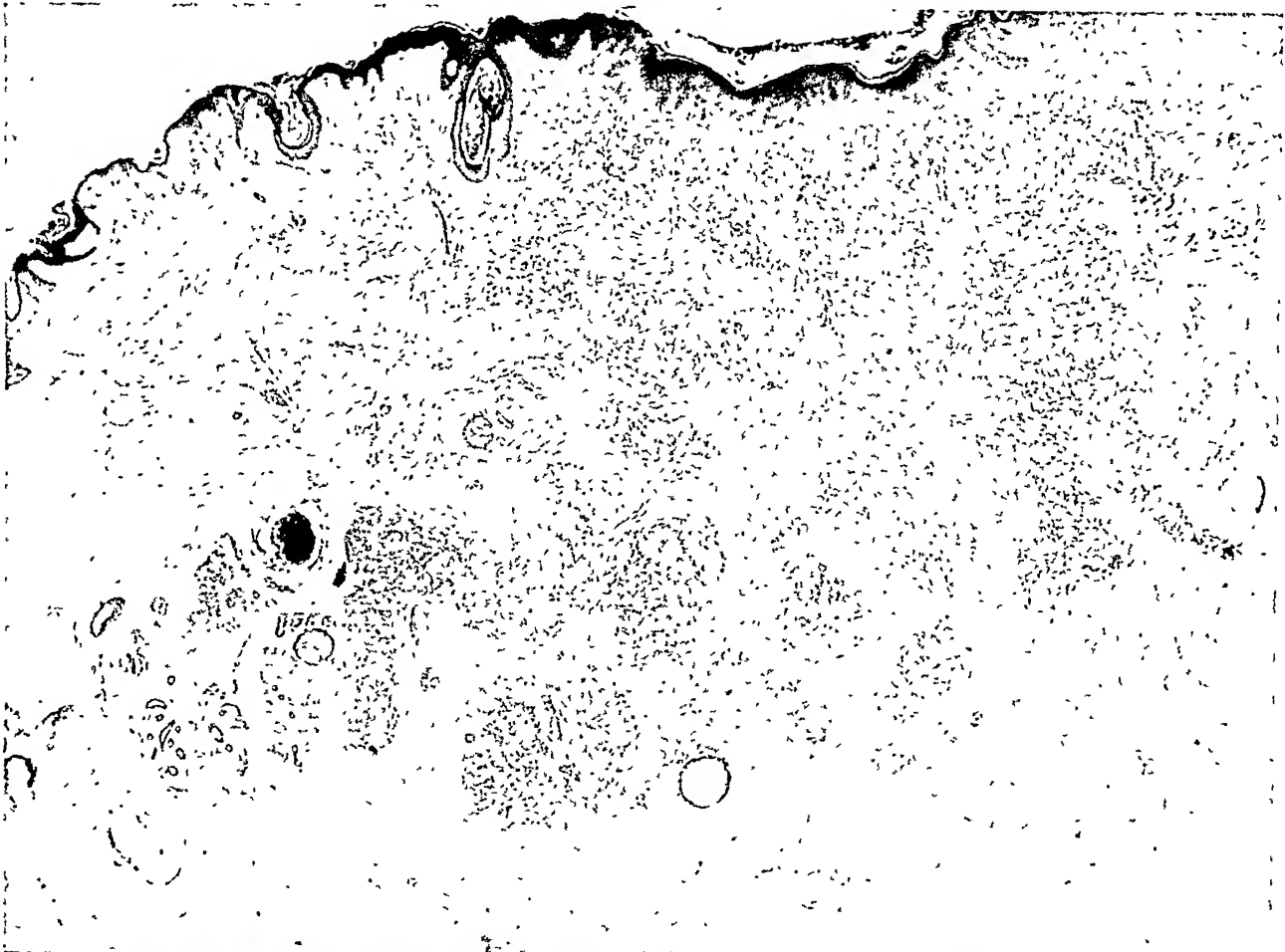
PLATE 75

FIG. 20. Reaction 10 months after a tick bite, showing dermal infiltrate with large lymphoid follicles that have led to erroneous diagnoses, particularly of lymphoblastoma. (A.I.P. neg. 90953.)

FIG. 21. Prominent lymph follicles forming part of a dermal reaction 10 months after a tick bite. (A.I.P. neg. 100440.)

FIG. 22. From the same case as Figure 19, showing a lymph follicle in relation to sweat glands. (A.I.P. neg. 90954.)

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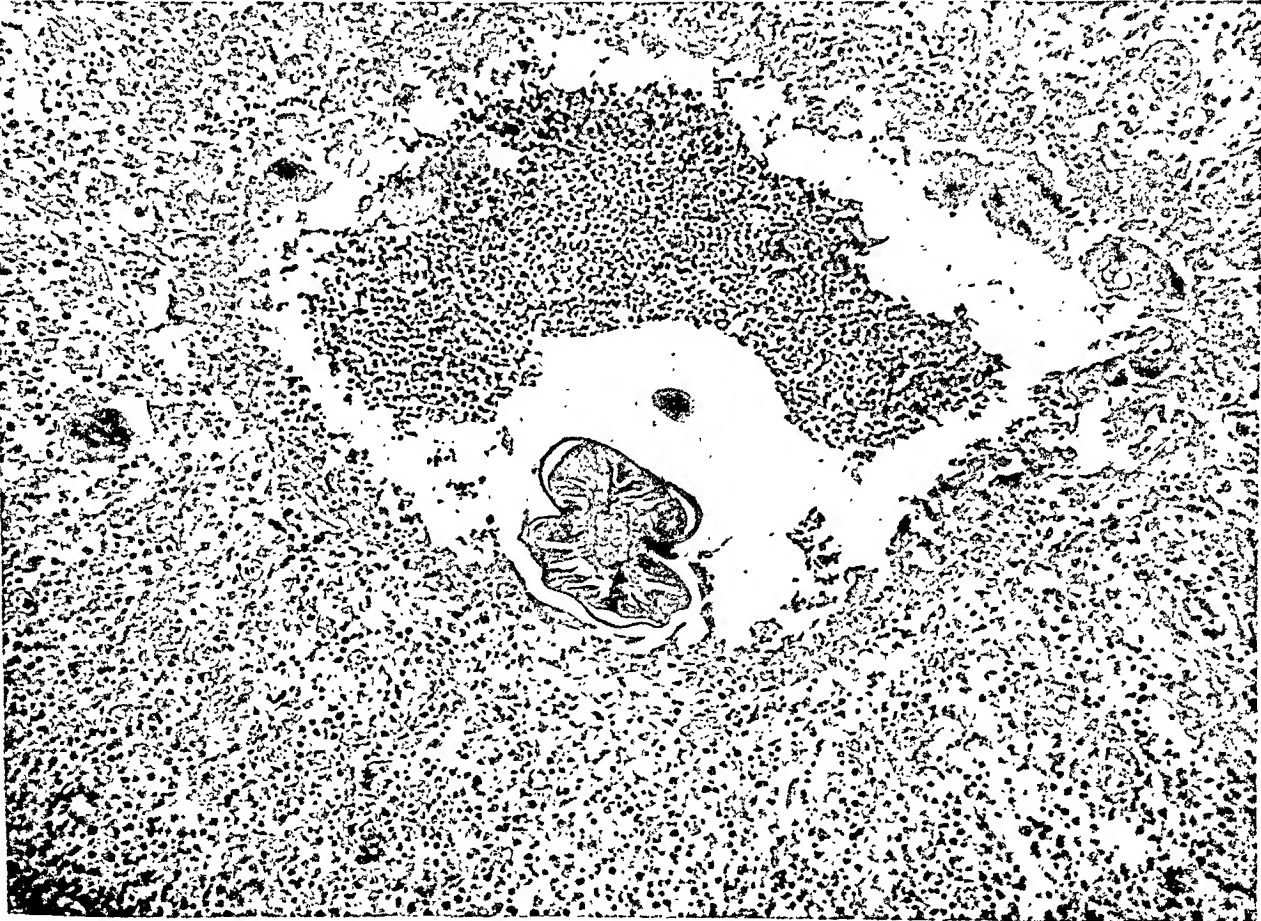
PLATE 76

- FIG. 23. Dermal "eosinophilic granuloma," a reaction to the ovum of *Schistosoma japonicum*, showing superficial scaling, the ovum, adjacent epidermal inclusion, and inflammatory reaction including numerous eosinophilic leukocytes. (A.I.P. neg. 89563.)
- FIG. 24. Dermal "eosinophilic granuloma," a reaction to the larva of *Ascaris*, showing numerous eosinophilic leukocytes and foreign body giant cells. (A.I.P. neg. 78694). (Courtesy of J. E. Ash and S. Spitz. Pathology of Tropical Diseases. W. B. Saunders Co., Philadelphia & London, 1945, 350 pp.)

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ALLERGIC HYPERGLOBULINOSIS AND HYALINOSIS (PARAMYLOIDOSIS) IN THE RETICULO-ENDOTHELIAL SYSTEM IN BOECK'S SARCOID AND OTHER CONDITIONS

A MORPHOLOGIC IMMUNITY REACTION *

GUNNAR TEILUM, M.D.

(From the Institute for Pathological Anatomy, University of Copenhagen, Copenhagen, Denmark)

Numerous investigations have shown a close relation between plasma cells and the pathologic globulins included in the gamma-globulin fraction and consisting chiefly of antibodies against different agents of the nature of antigens (Ranström). It may be stated here briefly that as early as 1913 Hübschmann made the supposition that plasma cells are able to produce antibodies; also, that Bing and Plum (1937) first emphasized the regular occurrence of plasma cells and other reticulo-endothelial cells in and outside the bone marrow in disorders associated with hyperglobulinemia, concluding that plasma cells are able to produce globulin themselves as has also been supposed more recently by a number of investigators.

A parallelism between hyperglobulinemia and accumulation of plasma cells in different organs, in particular in the spleen, has been demonstrated by investigations carried out by Björneboe and Gormsen (1943) on immunization of rabbits by means of polyvalent pneumococcal vaccine. These authors did not find an increase in plasma cells in hyperglobulinemia caused by injection of globulin. That hyperglobulinemia and increase of plasma cells should both be produced by the same cause, but should not otherwise be associated with one another, must be considered improbable after the demonstration by Bing, Fagraeus, and Thorell of the abundant content in the protoplasm of the plasma cell of ribose nucleotide. This substance is considered characteristic of the formation of proteins in cells, presumably giving rise to the basophilia of the cells and to their characteristic staining according to Unna's method. It has been supposed by Magnus-Levy and numerous other investigators that plasma cells in plasma cell myeloma produce the proteinic substances giving rise to hyperglobulinemia in myelomatosis.

In respect to many infections by well known bacterial agents, associated with hyperglobulinemia, it has been well established that the globulin is identical with antibody. It also has been possible during

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recent years to demonstrate hyperglobulinemia as a frequent and characteristic symptom in pathologic conditions of a more obscure nature. This applies to Boeck's sarcoid and to lupus erythematosus disseminatus. The cause of the hyperglobulinemia, its nature, and its relation to morphologic reactions have been unknown in these conditions. R. A. Moore in 1944 (page 545) stated that: "An unexplained increase of plasma globulin to 3 to 6 gm. per 100 cc. is a useful diagnostic sign" (in Boeck's sarcoid), and A. F. Coburn and D. H. Moore, in 1943 (page 213), said that "Hypergammaglobulinemia of unknown cause is a constant characteristic of disseminated lupus erythematosus."

In comparative pathologic-anatomic studies of Boeck's sarcoid, lupus erythematosus disseminatus, and other conditions, I have demonstrated, as a common feature in such disorders of different causation, a coincidence of hyperglobulinemia, paramyloidosis, or hyalinosis in the reticulo-endothelial system which is often decisive as a morphologic specific character, and is sometimes found in direct relation to an accumulation of plasma cells. In the fresh stages, precipitates of a homogeneous paramyloid substance are found, displaying transitional forms to hyaline deposits, frequently arranged in periarterial rings in the lymph nodes and the spleen. According to my observations, these changes must be considered phases of *an elementary morphologic immunity reaction with an underlying allergic hyperglobulinosis in the reticulo-endothelial system*.

The relation between these reactions in lupus erythematosus disseminatus will be described in a separate paper, while the findings in Boeck's sarcoid in particular will be discussed here. The elementary reactions, however, will be described first on the basis of a case of Letterer-Siwe's disease.

CASE I

Letterer-Siwe's Disease with Hyperglobulinemia, Paramyloidosis, and Accumulation of Plasma Cells in the Reticulo-Endothelial System

A boy, 9 years of age, was admitted to the Pediatric Ward of the University Hospital of Copenhagen (service of Dr. P. Plum) on May 6, 1946. He had had morbilli, pertussis, varicella, and rubeola. Following vaccination against smallpox at 2½ years of age, attacks of fever developed, with a rise of temperature to 41° C. of a few days' duration, the temperature then becoming normal; after a few months there was a constant increase of temperature, being normal in the morning but 38° to 39° C. in the evening. When he was 3 years old his mother observed a swelling of his right cheek and also swollen lymphatic nodes on the right side of his neck; he was found to be anemic at the same time (40 per cent hemoglobin). Since that time he had been constantly febrile, with a poor appetite and in bed for certain periods. The swelling of his cheek and of the lymphatic nodes persisted but varied much in size. In October, 1945, he had been admitted for 5 months to "Kronprinsesse Louises Barnsjukhus" in Stockholm, where a histologic examination of the enlarged lymphatic node from the angle of the jaw

gave a picture which most resembled that which is seen in eosinophilic granuloma of the bones or Letterer-Siwe's disease. Moreover, the serum albumin was found to be 1.92 per cent (the normal being 3 to 5 per cent); globulin, 6.26 per cent (the normal being 1.5 to 3 per cent); total protein, 8.18 per cent (the normal being 6.5 to 7.7 per cent); albumin/globulin ratio, 0.3 (normal, 1.5 to 2).

On examination in the Pediatric Ward of the University Hospital in Copenhagen the patient's face was found to be highly asymmetric, with considerable lateral swelling of the right cheek corresponding to the zygomatic bone. The infiltration was firm, not tender, measuring about 5 cm. in the vertical direction. Below the edge of the jaw there were lymphatic nodes. The skin showed nothing abnormal. The liver and spleen could not be palpated.

Roentgenograms of the skull showed considerable swelling of the soft parts on the right and a corresponding defect of the zygomatic arch posteriorly. There were no other alterations. The thorax, the extremities, and the pelvis displayed no abnormal signs roentgenologically. The sedimentation rate varied from 33 to 123 mm., being most frequently about 50; hemoglobin, about 60 per cent; leukocytes, 11,600 to 21,000; differential count, normal; eosinophils, 1 to 2 per cent; thrombocytes, 515,000. The Mantoux (Mendel) test was negative. No abnormal constituents were found in the urine. Total cholesterol was 175 mg. per cent; serum albumin, 3.78 mg. per cent; serum globulin, 5.11 mg. per cent; total protein, 8.89 mg. per cent.

During his stay in the hospital the patient was subfebrile for certain periods. He was discharged on June 30, 1946.

Histologic Examination

Histologic examination was based on the lymph node that had been excised in Stockholm and on subsequent biopsies of a lymph node of the neck and of the tumescence on the right cheek.

In a rather well defined area in the central part of a lymph node there was an accumulation of large, pale reticulum cells with abundant cytoplasm (Fig. 1), resembling the epithelioid cells of the granulomata in Boeck's sarcoid, but without tuberculoid structure. Especially in the peripheral parts of this area were found wide, very coarse bands of a homogeneous, partially hyaline substance, surrounded by a wide border of plasma cells which also passed in dense swarms between the individual bands (Fig. 2). As already mentioned, the latter were partially hyalinized, but in other parts they were of the nature of paramyloid and did not assume a blue but a red-violet color when stained according to Mallory's method. In the more peripheral parts of the lymph node there were finer trabecular and reticular homogeneous deposits in direct relation to plasma cells and reticulum cells. The vessels also contained a precipitate of homogeneous substance in many instances, the larger and smaller vessels being surrounded by concentric homogeneous rings (Fig. 3, for comparison with the alterations in the spleen in lupus erythematosus disseminatus), between which a few plasma cells and other reticulo-endothelial cells were observed.

There were no foam cells, nor giant cells. Microscopic examination of tissue of the tumescence in the right zygomatic region showed a

uniform structure with diffuse reticulum cell proliferation with close-set cells, not especially rich in protoplasm. Reticulin staining was positive; sudan staining, negative. No necrosis, eosinophilia, foam cells, or giant cells were found.

Histologic Diagnosis. Letterer-Siwe's disease.

Summary of Case 1

Letterer-Siwe's disease with a protracted course in a 9-year-old boy began with attacks of fever following vaccination against smallpox when the boy was 2½ years old. Microscopic examination of tissue from a focus in the zygomatic region showed diffuse reticulosis, while in the enlarged lymph nodes a large central accumulation of large, pale reticulum cells, rich in protoplasm, was found. The reticulum cells resembled the epithelioid cells in the granulomata in Boeck's sarcoid, circumscribed in the peripheral parts by coarse bands of homogeneous substance, in some parts hyaline, in others of the nature of atypical amyloid and surrounded by dense swarms of plasma cells. In addition, homogeneous precipitates were found around the vessels in the form of concentric homogeneous rings resembling the periarterial alterations in the spleen in lupus erythematosus disseminatus. There was also a marked hyperglobulinemia.

On close examination of the morphologic lesions in *Boeck's sarcoid*, all of the reactions described above can be found. However, while in the case of Letterer-Siwe's disease described here they reflect only the immunity conditions in this particular case, the analogous reactions in Boeck's sarcoid form the basis of the morphologic characteristics of this disease, illustrating the different phases of their development and explaining the nature of the hyperglobulinemia.

It has been established that cases of Boeck's sarcoid generally display an increase of the serum globulin. Salvesen (1935) first drew attention to this fact. He found an increase of the total protein content in the blood in 3 cases of Boeck's sarcoid owing to an increase of the globulin fraction with an albumin/globulin ratio of 0.86 to 0.51. Harrell and Fisher (1939) and Harrell (1940) found the total proteins to be over 8 gm. per cent in all but 3 of 11 cases which they studied, and the albumin/globulin ratio was reversed in all 8 cases during the active stage. Normal values were found in one of these after recovery. Bing (1940) found an increase of the serum globulin in 2 of 4 cases of Boeck's sarcoid. Fisher and Davis (1942) presented electrophoretic patterns for the sera of 12 cases of sarcoid, all of which had been proved by biopsy. In 4 cases which showed no clinical signs of activity

the sera were almost normal, there being only a slight decrease of albumin and increase of alpha globulin. Those with active lesions were found to have a marked elevation of the gamma globulin at the expense of the albumin, frequently with moderate hyperproteinemia. This electrophoretic pattern was similar to that which has been observed in association with the formation of antibodies in response to an infectious agent.

The morphogenesis and the phasic development of the characteristic lesions occurring in Boeck's sarcoid will be considered more fully in connection with the following case, partly on the basis of findings in post-mortem material and partly on the alterations of common occurrence in material taken for biopsy.

CASE 2

Boeck's Sarcoid with Marked Paramyloidosis

A woman, 28 years of age, was admitted to Medical Ward B of the University Hospital in Copenhagen (service of Dr. E. Warburg) on December 7, 1944. Her brother had died of pulmonary tuberculosis 9 years previously. The patient previously had been in good health. She was taken ill 1 year before admission with fatigue, loss of weight (15 kg.), functional dyspnea, and perspiration. She had not felt febrile and had had no pain in her side.

Examination. The patient was pale and lean. She had dyspnea when resting. Her tonsils were small. There was no glandular swelling in her neck, but one small gland was found in her left axilla and small glands were found in both inguinal regions. The thyroid gland was not enlarged. Upon examination of the heart, the apex impulse was felt in the fifth intercostal space inside the midclavicular line; there was no murmur; the 2nd pulmonic tone was extremely accentuated. Fine moist râles and crepitation were heard almost everywhere.

Roentgenologic examination of the lungs disclosed a large cavern in the left apex, passing through almost the entire depth of the lung. Below this there were a number of smaller caverns surrounded by fibrous adhesions. In the right lung also a large cavern was observed in the apex. On the whole, the lung was heavily infiltrated.

The temperature varied from 38° to 39° C. Hemoglobin was 95 per cent; sedimentation rate, 27; erythrocytes, 4.87 millions; leukocytes, 6,120; blood pressure, 90/40 mm. Hg. Urine: no albumin. Wassermann test of the blood, negative. No tubercle bacilli were found in repeated examinations of sputum. Direct microscopy and cultivation disclosed no tubercle bacilli. The Mantoux (Mendel) test was negative (all strengths, in repeated examinations).

On December 8 the serum protein was 7.1 per cent. Fractional determination of protein was not made.

On December 14, biopsy of the tonsil showed a tuberculoid structure (Boeck's sarcoid?). On December 19, biopsy of an inguinal gland showed a tuberculoid structure as in Boeck's sarcoid. The patient died on January 3, 1945.

Post-mortem Examination

Post-mortem examination showed the *bone marrow* of the vertebral column to be macroscopically unaltered. There were no alterations of

the *skin*. The *tonsils* were not enlarged and their cut surface was uniform. No ulcerations of the *larynx* or of the *trachea* was found.

The *pleurae* displayed extensive fibrous adhesions.

In the apex of the *right lung* a cavern was seen, measuring 3 to 4 cm. in diameter. Its walls were discolored, with irregular trabeculae and cords. The pulmonary tissue below was of nodular consistence and grayish red. The *left lung* also displayed a large cavern superiorly, measuring 6 cm. in diameter, with firm solidified areas in the walls. No miliary tubercles or peribronchitis were seen. The *bronchial glands* showed a large conglomeration of firm, only partially necrotic lymph nodes of a grayish red color, measuring 4 cm. in diameter.

The *heart* presented some hypertrophy of the right ventricle. Otherwise the myocardium was without alteration. The *lymph nodes* around the aorta formed large conglomerations of a firm consistence, having a pale grayish red cut surface. No necrotic areas were seen.

The surface of the *liver* was smooth; it was not enlarged and the cut surface was unaltered. The *suprarenal glands* showed no signs of tuberculosis. The *spleen* was enlarged, measuring 6 by 10 by 16 cm., with a dry cut surface and no tubercles. Its consistence was not so firm as in amyloidosis. The *ovaries and tubes* showed no signs of tuberculosis. The *brain and kidneys* were normal.

A *bacteriologic examination* was made (at the State Serum Institute) of a necrotic gland and the cavernous tissue of the right lung, of other tissue of the right lung, the spleen and a lymph node from the hilus. By this examination (comprising cultivation and inoculation into guinea-pigs) no signs of tuberculosis were demonstrated.

Histologic Examination

The *spleen* was permeated, as were the *lymph nodes*, by masses of typical epithelioid cells displaying a tuberculoid structure which was well defined. In most areas giant cells were few or absent. The picture was characteristic of Boeck's sarcoid. A very extensive hyalinosi (paramyloidosis) was especially remarkable, however, being localized partly to the peripheral parts of the epithelioid-cell granulomas, in which broad, homogeneous, concentric rings, often two to four, were found (Fig. 4), and partly to certain diffuse areas in the tissue (Fig. 5). The hyaline substance extended from the periphery into the individual granulomas, which were thus in many parts wholly replaced by hyaline masses. Around these more or less transformed cell accumulations, isolated homogeneous bands and clumps were found (Fig. 6), representing a more advanced phase of hyaline or paramyloid develop-

ment from the homogeneous "extragranulomatous precipitates" outside the tuberculoid structure, which in my remaining material of Boeck's sarcoid was a characteristic finding (Fig. 8).

In their central parts the tuberculoid structures contained typical double-contoured and stratified, often calcified corpuscles, described by Schaumann, among others, as being present in tonsils and lymphatic nodes in Boeck's sarcoid. A strikingly great number of plasma cells, in addition to other reticulo-endothelial cells, were found around the epithelioid cells. There was no necrosis, but in the central parts of the granulomas a precipitate of a homogeneous substance without cellular structure was observed in several spots. In the parts of the *spleen* in which the lesions were less extensive, the follicular arteries were found to be surrounded by broad rings of a hyaline substance resembling the typical lesions in lupus erythematosus disseminatus. In the peripheral parts of these rings granulomata often were found, surrounded by hyaline bands displaying a direct continuity with the periarterial rings. The concentric rings in the transformed granulomata also bore a close resemblance to the periarterial lamellae. Staining according to Mallory's method gave very fine pictures with rings of an intense blue from which a blue network passed between the intensely red epithelioid cells and into the reticulum tissue. Just as in case 1 of Letterer-Siwe's disease, red lamellae were found scattered among the blue bands of hyaline substance, such lamellae also being conspicuous in the deep part of the splenic capsule which displayed a high degree of hyaline thickening.

Jürgens' methyl violet reaction for amyloid and Congo red staining were negative.

Tubercle bacilli could not be demonstrated.

The *lymph nodes* also displayed typical Boeck granulomata with marked hyalinosis (paramyloidosis). Here, too, concentric homogeneous rings could be demonstrated around the smaller vessels (Fig. 7), reminiscent of the periarterial lesions in the spleen in lupus erythematosus disseminatus and of the alterations in a lymph node in case 1 of Letterer-Siwe's disease with hyperglobulinemia.

The *liver* contained scattered typical granulomata with a few giant cells. No necrosis was present.

The *lungs* displayed a highly variegated picture with well defined epithelioid cell granulomata surrounded by lymphocytes and in most instances by a hyalinized tissue, in some parts passing on to large structureless masses containing epithelioid cells and giant cells, a number of which resembled Langhans' cells. Others resembled regular

giant cells of foreign body type, frequently containing the stratified, often calcified, corpuscles referred to in the description of the spleen, which were stained a deep blue by hematoxylin and eosin. Some of the granulomata resembled those of Boeck, while others could not be distinguished with certainty from tuberculosis. In the walls of the cavern an even transition from a peculiar homogeneous necrosis to hyalinosis was observed. No tubercle bacilli were found on microscopic examination. Sudan staining showed no lipoid content in the granulomata.

The kidneys, suprarenal glands, thyroid gland, intestines, appendix, and the pituitary gland displayed no alterations, especially no tuberculous structure.

Summary of Case 2

Case 2 thus was noteworthy especially for the following reasons:

1. The combination of marked changes, like those seen in Boeck's disease (sarcoidosis), in the spleen, lymph nodes and lungs, with alterations in the lungs which, as far as the clinical features and the macroscopic picture are concerned, bore a close resemblance to tuberculosis (caverns), but without any tubercle bacilli being found on cultivation or microscopic examination, the tuberculin reactions also being negative. These findings may be made to correspond perfectly well with the prevailing Scandinavian view of Boeck's sarcoid as a form of tuberculosis with a high immunity (positive anergy, discussed later).

2. The combination of typical Boeck granulomata with paramyloidosis (hyalinosis), especially in the spleen, in which, in this connection, the close relation between the hyalinized border zone of the granulomata and the periarterial hyalinosis was noted in particular. This must be considered identical with the findings described above in Letterer-Siwe's disease with hyperglobulinemia, and with the lesions occurring in atypical and experimental amyloidosis, and in lupus erythematosus disseminatus.

DISCUSSION

The paramyloidosis in Boeck's sarcoid must be considered a definite phase of the development of the lesions, with an allergic hyperglobulinosis in the reticulo-endothelial system as the underlying primary cause. The paramyloid phase and the hyperglobulinemia in Boeck's sarcoid as well as in the other conditions mentioned must be considered an elementary immunity reaction in the reticulo-endothelial system.

In preparations from a number of other cases of Boeck's sarcoid, chiefly from lymph nodes, tonsils, and skin, I found good conformity with the view advanced here concerning the nature and phasic develop-

ment of the Boeck lesions, the morphologic structure of the lesions on the whole showing that a gradual development takes place from precipitate (Figs. 8, 9, and 10) to hyalinosi. The following features may be stressed in this connection:

1. Granulomata with large, pale epithelioid cells with *complete absence of necrosis*;

2. Outside the actual tuberculoid structure, precipitates of a homogeneous substance (*i.e., extragranulomatous precipitates*) of the same nature as that observed in the granulomata, staining in the same manner and forming homogeneous bands or clumps between the typical granulomata (Fig. 8);

3. A central homogeneous area in the granuloma, which is sometimes interpreted as slight or early necrosis, represents a similar precipitate, corresponding alterations also being observed in some instances in the walls of the vessels;

4. Preparations from cases of Boeck's sarcoid in the different phases of the disease show all transitions, from the precipitation of homogeneous eosinophilic substance (Figs. 8, 9, and 10) to marked *paramyloidosis* as in case 2, with broad concentric hyaline rings beginning peripherally in the individual granulomata (Fig. 4), which are gradually replaced by clumps of hyaline tissue (Fig. 6);

5. In most cases plasma cells in strikingly great numbers are found in direct relation to these paramyloid rings;

6. A marked periarterial hyalinosi in the *spleen* was localized to the follicular and the penicillary arteries, resembling the alterations in the spleen in lupus erythematosus disseminatus which will be dealt with in a subsequent publication.

Considering lastly the conformity with the findings in case 1 of Letterer-Siwe's disease (large, pale epithelioid cells without any necrosis; depositing of paramyloid beginning in the peripheral parts and in relation to the accumulation of plasma cells; extensive homogeneous and paramyloid deposits independent of the granulomata and giving the same staining reactions as the latter; vascular rings and hyperglobulinemia, which is a characteristic symptom in Boeck's sarcoid), I consider it highly probable that *the morphologic lesions in Boeck's sarcoid represent a serologic hyaline (paramyloid) precipitation, having as its starting point a globulin-precipitate, especially in the reticulo-endothelial system.*

In a number of preparations from different cases of Boeck's sarcoid stained according to Unna's method, the epithelioid cell granulomata and homogeneous precipitates during the active stage assumed a deep

red color, in contrast with the granulomata in tuberculosis. This must be considered as a further support of the view that a hyperglobulinosis is present.

The combination of Boeck's sarcoid and paramyloidosis, so conspicuous in case 2, requires special comment. This is not, as in tuberculosis with amyloidosis, a special complication but a *phasic development of the typical morphologic lesions* characteristic of Boeck's sarcoid. It now appears that the reactions described in Boeck's sarcoid and in case 1 of Letterer-Siwe's disease, like the increase of globulin in these conditions, are quite similar to the reactions previously demonstrated in "experimental amyloidosis" after immunization, so that the view of Boeck's sarcoid advanced above as an immunity reaction in which a *hyperglobulinosis* in the reticulo-endothelial system forms the basis of the morphologic lesions (reticulosis ending in paramyloidosis) as well as of the hyperglobulinemia, can easily be made to correspond with previous findings in such immunization experiments.

Hass, Huntington, and Krumdieck, in 1943, stressed that: "It seems, therefore, that persistent or repeated stimulation of immune mechanisms is a fundamental factor in the genesis of amyloid disease." The only exception stated is the type seen in plasma cell myeloma.

Loeschcke (1927) carried out sensitization experiments in rats with a 5 per cent solution of casein sodium and, soon after the first intraperitoneal injection, found a considerable increase in the volume of the spleen, with enlarged reticulum cells which were constantly increasing in number. He presumed that antigen-antibody reacted with one another, with the formation of an insoluble precipitate. All that is termed hyalin was said by him to be the morphologic expression of such antigen-antibody fixation, whereas amyloid was perceived as a special case of the serologic precipitation of hyalin, which is present primarily only at the place of formation of the antibody, *i.e.*, in the reticulo-endothelial system. Just as the specificity of the formation of antibody against different proteins is stressed, we must, according to Loeschcke, recognize specific forms of hyalin, of which, however, only amyloidosis is open to a histologic characterization. Still, it also applies in part to the atypical amyloidosis (paramyloidosis) described here and also to the depositing of amyloid in plasma cell myeloma, termed paraproteinosis (Apitz).

As early as 1926 Letterer pointed out hyperglobulinosis, *i.e.*, the increased liberation of globulin from the cells to the tissue sap and to the blood, as the primary basis of amyloidosis (*i.e.*, experimental amyloidosis), stating that it may be caused both by the known fundamental disorders and by protein therapy, and also emphasizing the

absolute, or at least relative, increase of serum globulin in such conditions. Lastly, mention may be made of the atypical amyloidosis in serum horses described by Arndt, and others, in which "the reticulo-endothelial cell reaction" seems to be of constant occurrence. Reticulo-endothelial reactions were present at all stages, when the animal had been immunized once, while amyloidosis occurred only when the animal had been used for the production of serum for 8 months at the earliest, and was fairly constant after 16 months. According to Arndt, such reticulo-endothelial alterations may appear as a precursory stage of amyloidosis, having in the spleen a typical *perinodular* localization in the splenic follicles; the serum horse also frequently displays very marked hyperglobulinemia.

With regard to pathogenesis, structure, phasic development (reticulum cell proliferation and precipitation), localization, and alterations in the blood, the points of resemblance between such forms of experimentally produced atypical amyloidosis and the findings in Boeck's sarcoid, as described here, are so striking that we have to reckon with completely parallel processes. The morphogenetic interpretation of the alterations in Boeck's sarcoid given above tells us nothing about the etiology of the disease; it may, however, easily be made to agree with the prevailing view of tuberculosis and Boeck's sarcoid as two phases of the same disease, supported as this view is by a number of publications (Lemming, Kallós, and Warfvinge, and others).

As has been pointed out by Hellerström, a special form of anergy is present in Boeck's sarcoid in all cases, which is fundamentally different from the anergy found in the organism that is not infected by tubercle bacilli. This type of reaction (of positive anergy) is doubtless the outcome of an especially high degree of immunity; "the organism tackles the tuberculin so rapidly that no reaction, or only a faint one, appears" (J. Jadassohn).

That the morphologic reactions dealt with here must be considered allergic immunity reactions is also illustrated by the unquestionable etiologic importance of the vaccination against smallpox in the case of Letterer-Siwe's disease reported as case 1. Also, in a case of typical, histologically verified Boeck's sarcoid, described by Lemming, in which the tuberculin reaction was negative, Boeck's sarcoid developed in the skin at the site of intradermic injection of B.C.G. vaccine, Mendel's reaction remaining negative after the injection.

While the described morphologic immunity reaction (hyperglobulinosis, paramyloidosis) in Boeck's sarcoid constitutes an essential part of the morphologic characteristics of that disease, similar reactions must also be supposed to play an important rôle in a number of other

disorders. This applies to lupus erythematosus disseminatus, which will be dealt with in the article that follows, and also to syphilis, lymphogranuloma inguinale (and certain cases of tuberculosis), in which specific "necroses" and hyalinosis, often in relation to plasma cell accumulation, are of frequent occurrence, hyperglobulinemia having been demonstrated also in such cases (Bing).

In these and in other special disorders associated with hyperglobulinemia, reactions analogous to atypical (and experimental) amyloidosis must henceforward be considered decisive for the morphologic specific pattern.

SUMMARY

As a feature common to Boeck's sarcoid and a number of other pathologic conditions associated with hyperglobulinemia, the reticulo-endothelial system is found to contain precipitates of a homogeneous substance passing on to hyalinosis (paramyloidosis). The alterations with regard to pathogenesis, structure, and phasic development (proliferation of reticulum cells and precipitation), localization, and alterations of the blood (hyperglobulinemia) must be considered analogous to atypical and experimental amyloidosis. The common primary basis is supposed to be an allergic hyperglobulinosis in the reticulo-endothelial system, determined by persistent or repeated stimulation of immune mechanisms.

In Boeck's sarcoid the following points, among others, are thus explained:

1. The localization in the reticulo-endothelial system.
2. The morphologic features (epithelioid-cell granulomata without any tendency to necrosis; the occurrence of "extragranulomatous" precipitates; the paramyloid phase with frequently concentric, hyaline rings in the border zone; the development of a periarterial hyaline zone in the spleen and in other organs, analogous to the periarterial fibrosis of the spleen in lupus erythematosus disseminatus).
3. The occurrence of hyperglobulinemia, which is a useful diagnostic sign in Boeck's sarcoid.
4. The state of immunity, in accordance with the generally accepted view of Boeck's sarcoid as a condition with a high immunity (positive anergy).

Like the different antibodies, various forms of hyalin and paramyloid must also, after these findings, be considered products of plasma cells and other reticulo-endothelial cells.

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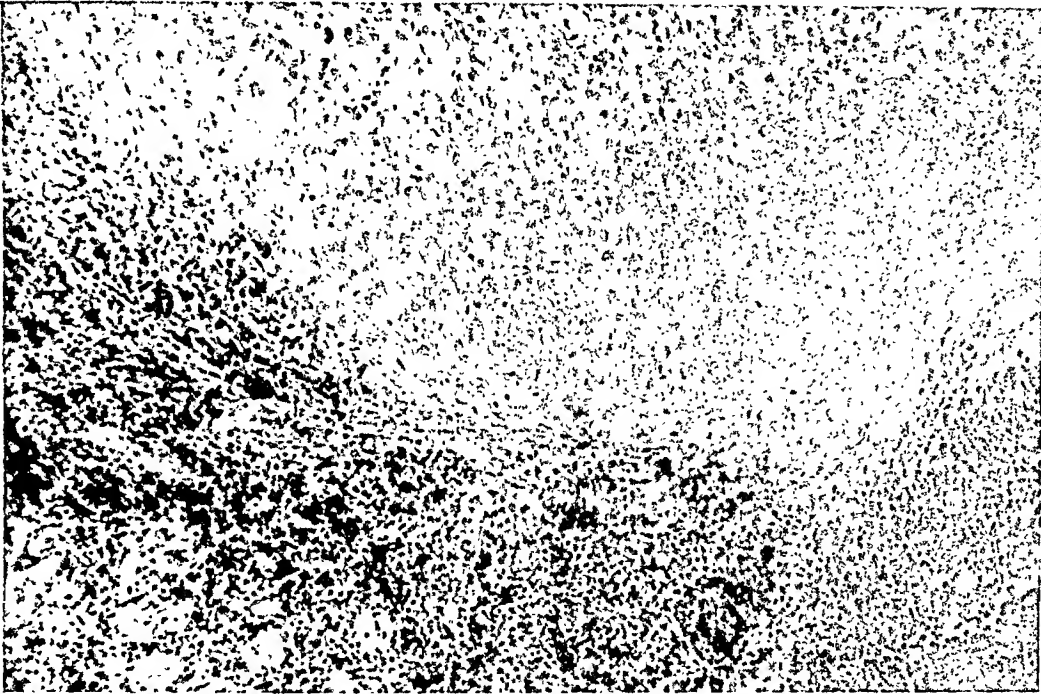
[Illustrations follow]

DESCRIPTION OF PLATES

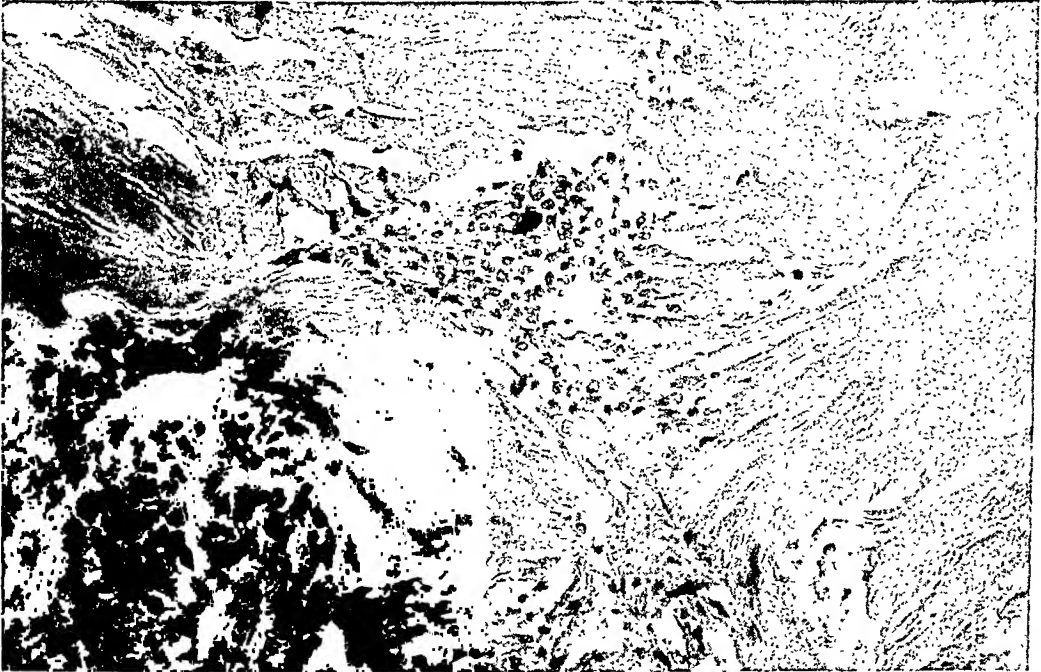
PLATE 77

- FIG. 1. Case 1. Epithelioid-cell reaction in a lymph node in Letterer-Siwe's disease with hyperglobulinemia. Hematoxylin and eosin stain. $\times 135$.
- FIG. 2. Case 1. Hyalinosis (paramyloidosis) with plasma cell accumulations in a lymph node. Hematoxylin and eosin stain. $\times 320$.
- FIG. 3. Case 1. Periarterial hyaline (paramyloid) rings in a lymph node. Hematoxylin and eosin stain. $\times 220$.

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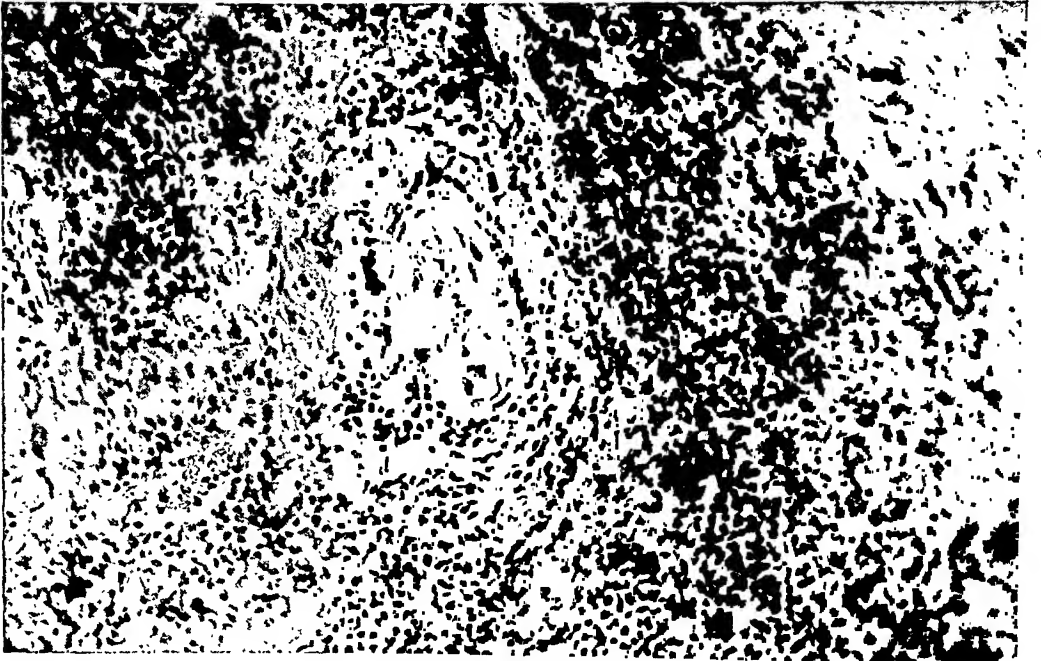


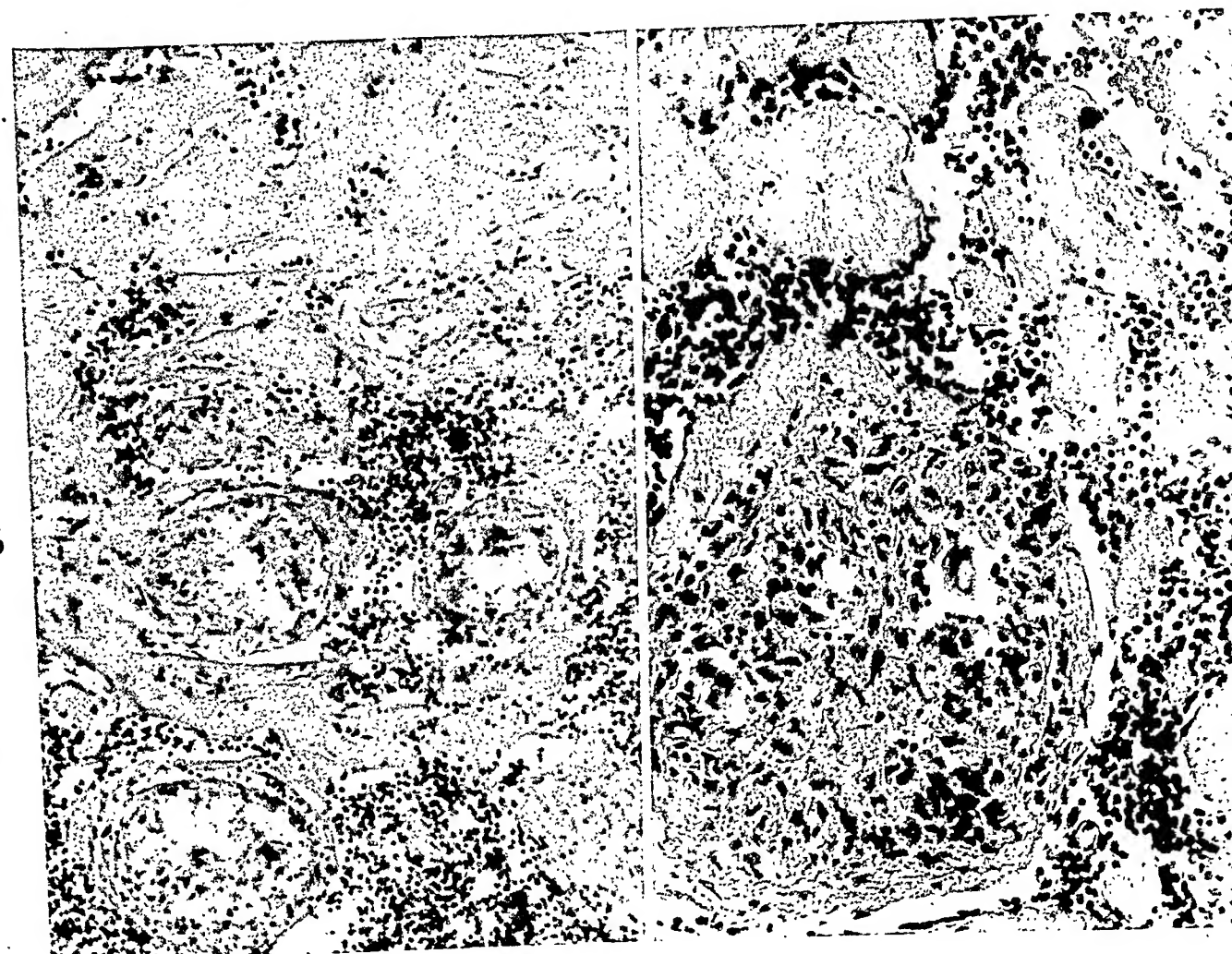
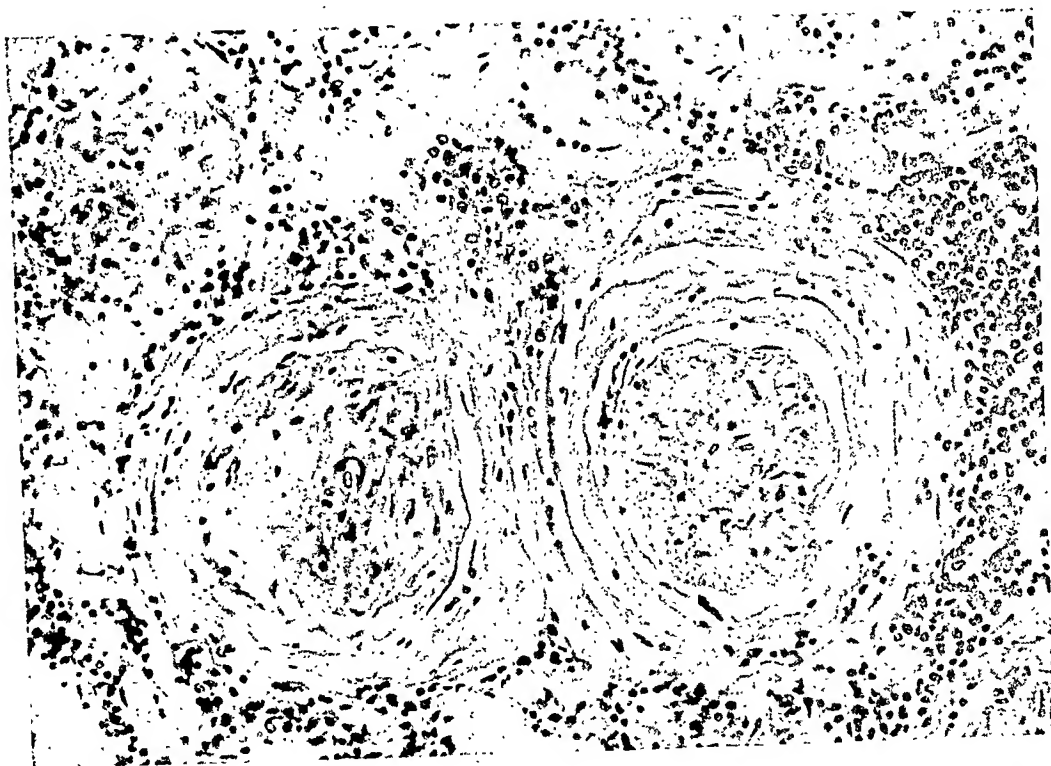
PLATE 78

FIG. 4. Case 2. Lymph node. Boeck granulomata with concentric paramyloid rings in the peripheral parts. Hematoxylin and eosin stain. $\times 220$.

FIG. 5. Case 2. Lymph node. Boeck's sarcoid, passing on to diffuse paramyloidosis. Hematoxylin and eosin stain. $\times 150$.

FIG. 6. Case 2. Boeck's sarcoid in spleen. Paramyloidosis beginning in the peripheral part of a granuloma. In the circumference isolated paramyloid bands and clumps are present. (For comparison with the extragranulomatous precipitates in Fig. 8.) Hematoxylin and eosin stain. $\times 320$.

4



Teilum

Allergic Hyperglobulinosis and Hyalinosis

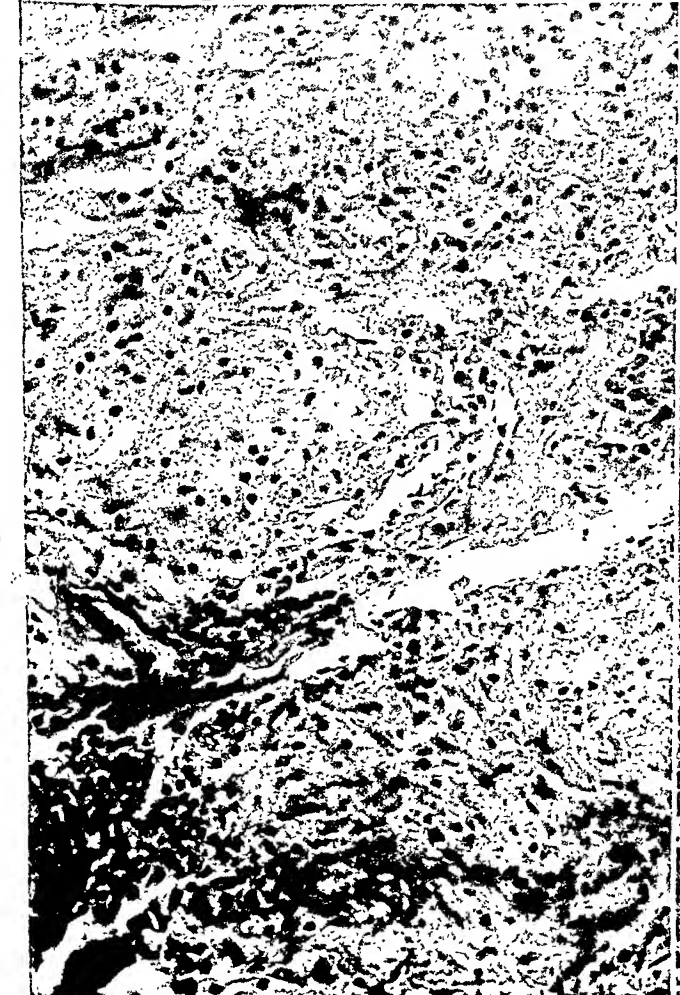
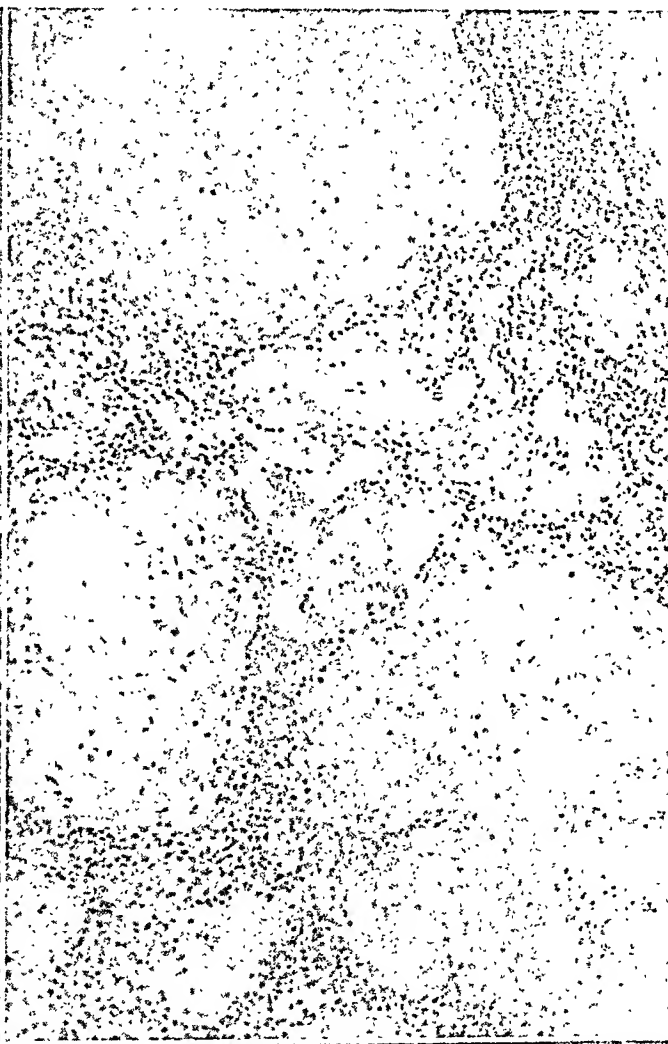
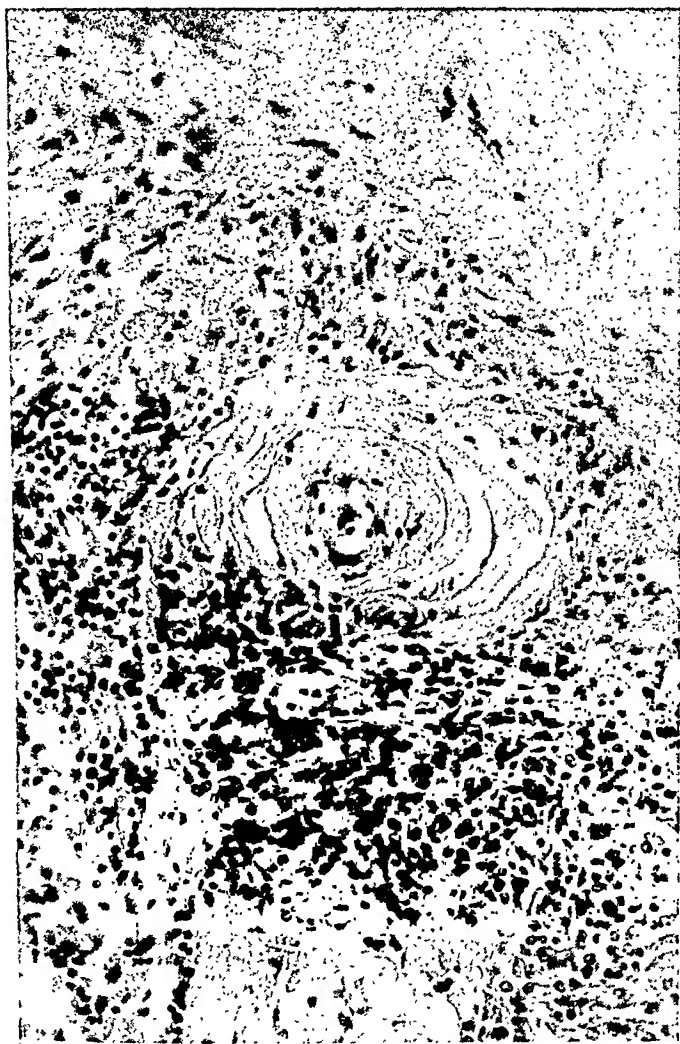
PLATE 79

FIG. 7. Case 2. Periarterial hyaline (paramyloid) rings in a lymph node in Boeck's sarcoid. (For comparison with Fig. 3.) Hematoxylin and eosin stain. $\times 220$.

FIG. 8. Extragranulomatous homogeneous precipitates (lymph node) without hyalinosis; a common finding in Boeck's sarcoid. (For comparison with Fig. 6.) Hematoxylin and eosin stain. $\times 200$.

FIG. 9. Extragranulomatous precipitates (lymph node, Boeck's sarcoid). Hematoxylin and eosin stain. $\times 240$.

FIG. 10. Lymph node in Boeck's sarcoid. Homogeneous precipitates assuming a red color when stained by Mallory's method. $\times 240$.



Teilum

Allergic Hyperglobulinosis and Hyalinosis

100

HYPERGLOBULINEMIA, PERIARTERIAL FIBROSIS OF THE SPLEEN, AND THE WIRE LOOP LESION IN DISSEMINATED LUPUS ERYTHEMATOSUS IN RELATION TO ALLERGIC PATHOGENESIS *

GUNNAR TEILUM, M.D.

(From the Institute for Pathological Anatomy, University of Copenhagen, Copenhagen, Denmark)

As a common feature of Boeck's sarcoid and a number of other conditions associated with hyperglobulinemia, I have described in the preceding article (1948) the precipitation, especially in the reticulo-endothelial system, of a homogeneous amyloid-like substance, passing on to hyalinosis, which, in the spleen and in lymphatic nodes *inter alia*, frequently occurs in the form of concentric rings. This is apparently an elementary morphologic immunity reaction with *an underlying allergic hyperglobulinosis in the reticulo-endothelial system*. That study emphasized the importance of this reaction in Boeck's sarcoid as an essential alteration pathogenetically related to the experimental "amyloidosis" observed after immunization. Those findings will be applied to lupus erythematosus disseminatus in the present study.

Investigations of recent years have shown that lupus erythematosus disseminatus very often is associated with hyperglobulinemia (Coburn and Moore, 1943, Thyresson, 1944) of unknown nature. Coburn and Moore (page 213) stated: "Hypergammaglobulinemia of unknown cause is a constant characteristic of disseminated lupus erythematosus."

In this disease the spleen, moreover, is the seat of a peculiar periarterial fibrosis confined to the central and penicillary arteries, which was first observed by Sacks (Libman and Sacks); it was described later by Klemperer, Pollack, and Baehr (1941) and Kaiser (1942), among others. This periarterial fibrosis, although not specific for lupus erythematosus, nevertheless is known to occur with a high frequency in this disease, as stated by Kaiser, who (page 38) further adds: "The connection between the periarterial fibrosis and the other lesions of disseminated lupus erythematosus is obscure."

It will be shown in the following study that the hyperglobulinemia and periarterial fibrosis occurring in the spleen in lupus erythematosus disseminatus are identical, pathogenetically and morphologically, with the alterations in Boeck's sarcoid and the atypical amyloidosis described in the preceding article (1948), having allergic hyperglobulinosis as a common primary foundation.

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*Allergic Pathogenesis and Morphogenesis of Lupus
Erythematosus Disseminatus*

The nature of the hyperglobulinemia and the periarterial fibrosis in the spleen in lupus erythematosus disseminatus must be considered in connection with the pathogenesis, which will therefore be mentioned here briefly. A discriminating survey of the different views of the nature of the disease, from 1872 to 1942, has been given by Libman who, in 1924, together with Sacks, described its special form of valvular and mural endocarditis. In a very thorough morphologic description by Klemperer, Pollack, and Baehr (1941), the alterations observed in lupus erythematosus disseminatus are considered "local manifestations of the widespread damage of collagen." These authors dissociated themselves from a concept of allergic pathogenesis, as they wrote: "Furthermore, the classic clinical evidences of hypersensitivity so frequently observed in periarteritis nodosa are lacking in disseminated lupus erythematosus. It would be a mistake, therefore, to speak glibly of lupus erythematosus disseminatus as an allergic disease—at least in the narrow sense of the term." The slight cellular reaction and the absence of a specific granulomatous phase are also stressed as contrasting with the ordinary findings in acute or granulomatous inflammation.

However, the miliary granulomata and nodular necrosis of focal occurrence in the serosa (parietal and visceral pleura), macroscopically visible as clustered grayish white nodules resembling miliary tubercles and described in my previous publications (1945, 1946), afford proof of the *allergic* nature of the disease; these alterations have to be interpreted as special phases of an allergic morphologic tissue reaction in conformity with the typical localization of the disease to the serous membranes. The focal allergic pneumonia described (1946), the endocarditis and the alterations in a number of other organs, such as lymph nodes, kidneys, vessels, and connective tissue, must henceforth also be regarded in the same way and may, in certain of their phases, be completely parallel with Arthus' allergic necrosis (1946). With regard to the Arthus phenomenon, reference may be made to Culbertson's demonstration (1935) of the fact that it is clearly dependent on the occurrence of circulating antibody, thus being of the same nature as anaphylaxis. After neutralization of the circulating precipitin, the phenomenon could no longer be elicited. Apart from all differences, I now see obvious pathogenetic points of resemblance with the conditions dealt with more fully in the preceding article (1948), Boeck's sarcoid and the morphogenetically related experimental atypical amyloidosis, in which "persistent or repeated stimulation of immune mechanisms is a fundamental factor in genesis."

Hyperglobulinemia in Lupus Erythematosus Disseminatus

Both in the conditions mentioned above and in lupus erythematosus disseminatus an increase of the serum globulin is found. In the latter disease, this is a characteristic symptom of frequent occurrence. It was first pointed out by Coburn and Moore (1943), who found an inversion of the albumin/globulin ratio in 15 patients, all of them being females between 6 and 36 years of age. In most of the cases the total protein was within normal limits. Each albumin determination was below the normal (mean value, 3.1), whereas each patient had a total globulin above the normal (mean value, 3.8) and each euglobulin value was found to be considerably increased (mean value, 1.0).

Electrophoretic analyses showed that this increase of globulin was to be found chiefly in the gamma fraction with an abnormally low albumin/globulin ratio. The other globulin fractions were approximately normal. Among other conditions in which such abnormally large quantities of gamma-globulin have been demonstrated, mention is made of the serum of hyperimmune antipneumococcal horses, Boeck's sarcoid, lymphogranuloma venereum, and certain cases of plasma cell myeloma.

Periarterial Fibrosis of the Spleen in Lupus Erythematosus Disseminatus

The high frequency of periarterial fibrosis of the spleen in disseminated lupus erythematosus is apparent from the literature (see Kaiser, page 31). Klemperer, Pollack, and Baehr (1941, 1942) ascertained its presence in 19 of 20 cases; Kaiser, in 15 of 18 cases.

Klemperer, Pollack, and Baehr (1941, 1942) described this lesion as a special periarterial fibrosis confined to the central and penicillary arteries, which in cross sections display concentric rings consisting of thick collagenic fibrils produced at the sacrifice of periarterial lymphatic tissue. In one of their cases many of the newly formed collagenic fibrils showed signs of fibrinoid degeneration, eosinophilic swelling and homogenization, and even basophilia.

Kaiser found macroscopic perisplenitis in 10 of 18 cases and considered periarterial fibrosis present when the periarterial collagen of the follicular and penicillary arteries, which normally is closely packed and without evidence of hyalinization, was found to be present in at least three layers, around at least half the circumference of the vessels, producing the appearance of concentric rings. Kaiser stated that this collagen was hyaline in most cases, and in others it was partially broken down to granular eosinophilic material. In most instances the majority of the smaller arteries were affected, whereas periarterial fibrosis was not found in relation to the medium or large arteries, or

around any of the venous structures. As a consequence of the fibrosis, the average diameter of the follicular vessels was found to have increased to about $125\ \mu$ in the cases affected, as compared to the normal average of 60 to $75\ \mu$.

Periarterial Hyalinosis in Allergic Conditions Associated with Hyperglobulinosis

After the demonstration of similar hyaline periarterial alterations in the spleen in lupus erythematosus disseminatus and in various other disorders, I have arrived at the view that the periarterial fibrosis in lupus erythematosus disseminatus is not specific; it is essentially different from the focal allergic reactions with necrosis and granuloma formation described in the latter disease. It is a lesion of the spleen which is common to a number of different disorders which have in common the feature of displaying an *allergic hyperglobulinosis* of the reticulo-endothelial system with persistent or repeated stimulation of immune mechanisms as the fundamental factor in the genesis.

a. Lupus Erythematosus Disseminatus

In my previous description (1945, 1946) of the allergic lesions in the narrowest sense of the term (miliary granulomata and nodular foci of necrosis) which occur in lupus erythematosus disseminatus, the alterations of the spleen were not mentioned. Both of the cases reported (1945), however, displayed a typical periarterial hyaline zone with concentric rings. In *case 1* (no. 366/44) the follicular and penicillary arteries of the spleen were thus found to be surrounded by concentrically arranged hyaline bands (Fig. 1) forming up to five lamellae which caused the external diameter of the vessels to be more than twice normal. The individual rings were composed of large and small, often slightly wavy, disconnected hyaline lumps which assumed a red color when stained according to the van Gieson-Hansen method, and a deep blue color with Mallory's stain. When the latter staining method was employed, the vascular wall itself was seen to contain an intensely red-colored substance, and in the parts peripheral to the latter a few coarse, red, Mallory-stained filaments could be observed in relation to the blue hyaline bands, small red bands also being seen peripherally or between the hyaline rings. In the circumference and scattered between the hyaline rings numerous reticulo-endothelial cells were seen, including a few plasma cells which assumed an intensely red color when stained according to Unna's method. The hyaline substance did not give an amyloid reaction with Jürgens' methyl violet or Congo red staining.

Case 2 of lupus erythematosus disseminatus (no. 447/44) displayed

a similar picture (Fig. 2). Here, too, scattered reticulo-endothelial cells were observed around and between the hyaline rings. With regard to the structure of the periarterial fibrosis of the spleen in lupus erythematosus disseminatus, reference may also be made to the more detailed descriptions in the articles by Klemperer, Pollack, and Baehr and by Kaiser.

b. Periarterial Hyalinosis of the Reticulo-Endothelial System Associated with Allergic Plasma Cell Reaction

In various previous observations (Bing and Plum, Björneboe and Gormsen) a connection between an accumulation of plasma cells and other reticulo-endothelial cells and hyperglobulinemia in different pathologic conditions and immunization has been demonstrated, a fact greatly supporting the supposition that these cells are themselves able to produce globulin. The allergic hyperglobulinosis and hyalinosis (paramyloidosis) described in the preceding article (1948) as occurring in the reticulo-endothelial system and which I have considered as the basis of the alterations dealt with here must, however, be associated with this property of the cells. It now appears that whereas large accumulations of plasma cells do not seem to be of common occurrence in the reticulo-endothelial system in lupus erythematosus disseminatus, a periarterial fibrosis of the spleen and of lymph nodes, completely corresponding to the alterations in lupus erythematosus disseminatus, may be accompanied in other conditions with allergic hyperglobulinosis and hyperglobulinemia by a marked accumulation of plasma cells. In some cases an *allergic plasmacytosis* of this nature may dominate the clinical and the pathologic-anatomic pictures and may in a number of cases give rise to confusion with plasma cell myeloma or aleukemic plasma cell leukemia.

Case 3

The patient was a man, 69 years of age, who previously had been in good health, but was admitted to the hospital because of an affection of the skin resembling psoriasis. It was of 1 year's standing and, after admission, appeared as a typical lupus erythematosus. Otherwise there were no special findings in the objective examination. Sedimentation test: 104, 110, 100 mm.; Takata's test, positive; serum protein, 9 per cent; albumin/globulin, 2.2 per cent/6.8 per cent = 0.32; thrombocytes, 150,000; hemoglobin, 70 per cent; erythrocytes, 3.8 millions; color index, 0.83; leukocytes, 3,000. Differential count: segmented forms, 44 per cent; lymphocytes, 32.5 per cent; monocytes, 20 per cent; eosinophils, 2.5 per cent; basophils, 1 per cent. Urine: 1 plus albumin; Wassermann's test on blood, negative; blood pressure, 140/70 mm. Hg; blood urea, 67 mg. per cent increasing to 331 mg. per cent. Temperature up to 37.6° C. The patient died in uremia.

Post-mortem examination (no. 204/42) revealed, in addition to a right-sided lobar pneumonia and fibrinous pleuritis, alterations of the

kidneys with rough, flat depressions on the surface and irregular narrowing of the tissue border. The consistence was increased; the vessels were gaping. The right kidney was slightly larger than the left.

Histologic examination showed a chronic pyelonephritis and an extremely marked periarterial fibrosis in the spleen (Fig. 3), all follicular and penicillary arteries being surrounded by very broad, concentric, hyaline rings. In contrast with the usual findings in lupus erythematosus disseminatus, extraordinarily well defined plasma cell infiltrates (which stained according to Unna's method) were found around the hyaline rings and between the single lamellae of the latter. When Mallory's staining method was used, red lamellae were observed here and there between the others that had been stained blue. Jürgens' methyl violet stain and Congo red staining for amyloid each gave negative results.

A coincidence of hyperglobulinemia, periarterial fibrosis of the spleen, and marked plasmacytosis in the spleen was thus found in this case. There were no findings in support of a diagnosis of plasma cell myeloma or aleukemic plasma cell leukemia, in which periarterial fibrosis of the spleen is not seen either. Consequently, I considered this case to be one of allergic plasma cell reaction in the reticulo-endothelial system, with hyperglobulinosis and periarterial hyalinosis in the spleen, also with hyperglobulinemia.

The reactions in this case are similar to those of case 1 of *Letterer-Siwe's disease* reported in the preceding article (1948). The findings in that case were simultaneous hyperglobulinemia and hyalinosis (paramyloidosis). The concentric homogeneous rings surrounding the vessels (see Fig. 3) and marked accumulation of plasma cells are also similar.

In conformity with the common features of a number of conditions with stimulation of immune mechanisms, the periarterial fibrosis of the spleen observed in lupus erythematosus disseminatus can be considered morphogenetically parallel in all respects to the alterations found in Boeck's sarcoid, in so-called genuine amyloidosis (and paramyloidosis), and also in experimental amyloidosis after immunization; *i.e.*, it is a morphologic immunity reaction in the reticulo-endothelial system.

Examination of preparations from the case of *Boeck's sarcoid* in which post-mortem examination was made and which was reported in the preceding article (1948) revealed a marked periarterial fibrosis of the spleen (Fig. 4), in many places in close relation to the hyalin (paramyloid) developed around the epithelioid cell granulomata. In this case, too, numerous plasma cells and other reticulo-endothelial cells were observed between the lamellae. The hyaline deposits did

not stain like amyloid with Congo red, but with Mallory's staining method numerous fine and coarser red bands were found in the hyaline tissue that had been stained blue. Another striking feature is the resemblance between the concentric paramyloid rings in the peripheral parts of the granulomata in Boeck's sarcoid (Fig. 4 of the preceding article) in which numerous plasma and reticulum cells were embedded and the hyaline periarterial rings found in lupus erythematosus disseminatus.

Pathology of Lupus Erythematosus Disseminatus

The periarterial hyalinosis referred to here takes a position of its own among the pathologic-anatomic alterations in lupus erythematosus disseminatus, partly owing to its constant localization, partly because of the sclerosing nature of the process. In these respects it is in contrast to the local changes observed in such cases in which there is evolution by stages with total, possibly fibrinoid, allergic necrosis, development of miliary granulomata, and secondary fibrosis (1945, 1946).

Another lesion of frequent occurrence and constant localization in lupus erythematosus disseminatus is the special alteration of the glomerular coils of the kidneys which has been previously characterized as "wire loops" because of the resemblance of the coils to bent wire (Baehr, Klemperer, and Schifrin). This is generally considered the most striking alteration of the kidney in this disorder. According to Klemperer, Pollack, and Baehr (1941, 1942), the coils are irregularly thickened and rigid, and are strongly eosinophilic. The thickening is found between the endothelium and the epithelium, apparently in relation to the basal membrane, resembling amyloid but failing to respond to all staining reactions for amyloid. In other cases, as in my case 1 (1945), there is *focal* fibrinoid necrosis of part of the glomerular coils, the others remaining unaffected. There seems to be no gradual transition between these two forms of alteration.

While the foci of necrosis of the glomerular coils are naturally considered analogous to the other scattered, allergic, in the narrowest sense, lesions, the "wire loop lesion" seems to be essentially different and its nature can probably be explained on the basis of the above-mentioned observations. For, if we grant that in such disorders as lupus erythematosus disseminatus, Boeck's sarcoid and atypical (genuine) amyloidosis there is, at any rate at certain stages, a hyalinosis of the reticulo-endothelial system (especially of the spleen), in addition to a stimulation of immune mechanisms with hyperglobulinemia, it seems natural to accept the "wire loop lesion" as a hyalinosis analogous to the deposition of amyloid in the kidneys. Like Loeschcke, we may here reckon

with specific forms of hyalin, of which only the amyloid is open to a histologic characterization. In "paramyloidosis" the amyloid reactions are not constant either.

A relation between the wire loop lesion and the changes in so-called genuine amyloidosis may be illustrated by the following case.

Case 4. Polyarthritits Chronica with Atypical Amyloidosis

The patient was a woman, 63 years of age, who had been admitted several times to Department A of the University Hospital of Copenhagen, service of Professor C. Sonne, for polyarthritits chronica rheumatica. She had never been affected with rheumatic fever, but had had angina tonsillaris several times a year and also recently. The onset of the patient's rheumatic disorder, in 1922, was associated with angina tonsillaris in the course of which she developed pain and swelling of the ankle joints, later spreading to other joints. About 18 months previously, she had had angina, fever, and albuminuria. Since then her condition had varied, but albuminuria had been present constantly. About 1 month before admission she had articular pain with increase of temperature to 39° C., in connection with influenza.

On examination a systolic murmur was found over the entire precordium in addition to swelling and tenderness of the joints. Blood pressure was 140/110 mm. Hg; hemoglobin, about 70 per cent; erythrocytes, 3.60 millions; leukocytes, 7,800; differential count, 61.5 per cent segmented forms, 4 per cent eosinophils, 1.5 per cent basophils, 25 per cent lymphocytes, 8 per cent monocytes. Wassermann test of the blood, negative; complement deviation reaction for gonococcal antibodies, negative.

On her first admission in 1942 the sedimentation test was from 34 to 91 mm. in 15 examinations made at regular intervals. On her second admission (from February 6 to May 1, 1946) it was found to be increasing evenly: Feb. 7, 82; Feb. 11, 91; Feb. 23, 92; March 2, 117; March 9, 107; March 13, 117; April 3, 144; April 13, 154; April 20, 140 mm. At the same time there was increasing *albuminuria* (up to 20 gm. per liter), increasing *blood urea* values (Feb. 7, 23; April 3, 82; April 13, 90 mg. per cent), and increasing *serum globulin* values:

	Total protein per cent	Albumin per cent	Globulin per cent
2/11/46	6.0	3.2	2.8
3/13/46	6.4	2.9	3.5
3/16/46	6.0	2.7	3.3

On her first admission, in 1942, the antistreptolysin titer was found to be increased (200); later it was normal.

Post-mortem examination (no. 212/46) showed pronounced brownish pigmentation of the skin, especially of the parts normally exposed to light. The *liver* was of normal size, with slightly increased consistence and giving a faintly positive amyloid reaction (iodine-potassium iodide). The *spleen* measured 15 by 8 by 4 cm., weighed 280 gm.; the cut surface was firm, elastic, and translucent. The *kidneys* were of normal size, displaying a slight granulation of the surface. The cortex had narrowed, and was pale. Spleen, kidneys, and suprarenal glands gave an intense amyloid reaction. Post-mortem diagnosis: Progressive polyarthritits; amyloidosis, marked in spleen, kidneys, and adrenals,

and mild in liver and small intestines; emaciation; melanoderma; bilateral hydrothorax; Addison's disease due to amyloidosis(?).

Histologic examination. In the periarterial zones to which the fibrosis is localized in lupus erythematosus, the *spleen* displayed homogeneous rings which assumed a purple-red color when stained according to Jürgens' method for amyloid. A similar substance was demonstrated in the intima, and in homogeneous clumps surrounding the vessels. There were numerous plasma cells.

The *kidneys* displayed marked glomerular alterations, with deposits of homogeneous substance between endothelium and epithelium in the coils, not so massive as they are normally seen in amyloidosis, but bearing a close resemblance to the wire loop lesion (Fig. 5). The substance assumed a blue color with Mallory's stain. With Jürgens' method it gave a faintly positive amyloid reaction. Congo red staining was negative. The amyloid and numerous casts in the tubules assumed a red color when stained according to Unna's method. The walls of the arterioles also contained large deposits.

In the course of a comparatively short time this patient presumably developed an atypical amyloidosis simultaneously with a highly increasing sedimentation reaction and an increase of the serum globulin. The spleen contained homogeneous deposits around the follicular arteries, in the kidneys deposits were found bearing a close resemblance to "the wire loop lesion" in lupus erythematosus disseminatus, and numerous homogeneous cylinders were present in the tubules staining in the same manner and presumably representing deposits of a globulin-like substance.

The underlying common immunity reaction was described in the preceding article (1948). Mention may also be made here of Stoeber's description (1934) of cases of allergic conditions combined with so-called genuine amyloidosis, and of Cazal's case of amyloidosis in a 7-year-old girl with "un état d'anaphylactique" as the only etiologic factor. It appears to me to be natural to associate both the splenic periarterial hyalinosis and "the wire loop lesion" in lupus erythematosus disseminatus with the allergic hyperglobulinosis of the reticulo-endothelial system after the analogy of the conditions in atypical amyloidosis and paramyloidosis. In this way "the wire loop lesion" of the kidney would have to be considered a *glomerulonephrosis* like glomerular amyloidosis, consisting in the deposition of a globulin product between the endothelium and the epithelium in the capillary coils. Special immunobiologic conditions as well as the time factor must be supposed to be of importance in the development of the alterations and to their degree in the individual cases. It may be mentioned here that in the case of lupus erythematosus disseminatus previously de-

scribed by me (1945), and not displaying any signs of the wire loop lesion but widespread focal allergic lesions with necroses and development of granulomata, the symptom of hyperglobulinemia was lacking.

A distinction presumably should be made between different phases of the disease. A whole series of lesions which are allergic in the narrower sense (Teilum, 1946) are thus pathogenetically related to periarteritis nodosa, arteriolitis granulomatosa allergica, and to some extent resemble allergic reactions in serum sickness and experimental sulfathiazole intoxication described by Rich (1942) and in allergic syndromes of other nature (Bergstrand). In other cases the deposition of coagulable material resembling amyloid is the predominant feature, especially in the spleen and the glomeruli, but presumably also in the walls of the vessels. These last mentioned cases thus display morphogenetic features in common with "genuine" or atypical, and also experimental amyloidosis, and with the conditions with hyperglobulinemia, in particular Boeck's sarcoid, mentioned in the preceding publication. It is possible that we may in certain cases distinguish between an anergic form (positive anergy), in conformity with the conditions found in Boeck's sarcoid, and an allergic form in the narrower sense of the term, with preponderance of necrosis, possibly with cellular resorption and development of granulomata (Teilum, 1946).

Besides the relation already described between lupus erythematosus disseminatus and other allergic conditions (Teilum, 1946), a certain connection with such conditions as scleroderma, purpura haemorrhagica, and dermatomyositis (see Libman) also has been proposed. The connection between hyperglobulinemia, alterations in spleen and kidneys in lupus erythematosus disseminatus, and genuine and experimental amyloidosis seem to elucidate these conditions.

The occurrence of amyloid nodes in the skin has thus been demonstrated in clinical scleroderma (Lubarsch, case 1). In Goetz' case of generalized scleroderma there was thickening of the intima in the blood vessels with "fibrinoid" material and alterations resembling periarteritis nodosa, a "marked thickening and increase of the perivascular connective tissue" being demonstrated in the spleen. In a case clinically resembling Goetz' and described by Jörgensen, dermatomyositis-like alterations were found to be combined with widespread paramyloidosis and increase of plasma cells in the sternal marrow (20 per cent). The simultaneous occurrence of periarteritis nodosa and atypical amyloidosis has been described by Volland, and in "serum" horses with amyloidosis Doerken often found allergic intimal granulomata in the hepatic veins, resembling the granulomata of endophlebitis hepatica.

With regard to purpura hæmorrhagica, reference may be made to

the clinical picture of "purpura hyperglobulinemica" established by Waldenström, but purpura also has been described several times in atypical amyloidosis (e.g., Lubarsch, case 2) in which many plasma cells were found in the spleen. Like hyperglobulinemia, purpura is also a symptom of frequent occurrence in lupus erythematosus disseminatus. Especially in cases of atypical amyloidosis with hyperglobulinemia the accumulation of plasma cells may be very considerable. Such a case which, like my case 3, might easily be considered one of plasma cell leukemia but for that accumulation, will be reported here.

Case 5. Hyperglobulinotic (Paramyloid) Syndrome Caused by Sulfonamides

A man, 58 years of age, was first admitted to Department A of the University Hospital of Copenhagen, service of Dr. C. Sonne, for examination because of albuminuria, ascertained 1 week before. He had no hematuria, hypertension, or immediately preceding infection. No previous renal disorder had been recognized. He had recently been suffering from fatigue and for the past 3 weeks from edema of the legs. Seven months previously he had had right-sided pneumonia with a protracted course in spite of treatment with sulfonamide (100 gm. in all). Pleuritis developed. When admitted for the second time he had constant albuminuria, about 12 gm. per liter. The blood showed a simple anemia. Hemoglobin had decreased from 96 to 55 per cent, and the sedimentation rate was increasing, from 50 to 100 mm. The formol-gel test was negative. There was no Bence-Jones protein in the urine. On Aug. 7, 1944, the total serum protein was 5.16 gm. per cent, with albumin, 2.36 gm. per cent, and globulin, 2.80 gm. per cent; on Dec. 19, 1945, serum protein was 7.5 gm. per cent, albumin, 3.10 gm. per cent, and globulin, 4.4 gm. per cent. Blood pressure varied from 90-125/50-90 mm. of Hg. Blood urea determinations were: Oct. 10, 1944, 40 mg. per cent; Dec. 11, 1945, 144 mg. per cent; Dec. 15, 1945, 156 mg. per cent. The patient died in uremia.

Post-mortem examination (no. 481/45) showed a widespread fibrous pericarditis. The heart measured 11 by 11 cm., the weight being 440 gm. The wall of the left ventricle was 17 mm. thick. The myocardium was pale, without any macroscopic signs of fibrosis or myomalacia. Valves and ostia were normal. The aorta showed some atheromatosis. There was no ascites. The stomach displayed multiple fresh erosions. The colon showed no ulcerations. The liver measured 24 by 16 by 10 cm.; the surface was smooth and pale, and the cut surface had normal markings. The organ was of normal consistence. The pancreas and the suprarenal glands were normal. The spleen was enlarged, measuring 6 by 9 by 16 cm. Its cut surface was reddish and of a gritty consistence. The kidneys measured 3 by 5 by 11 cm., being slightly decreased in size. The surface displayed extensive, irregular, rather coarse depressions. The color was mottled deep red and pale red. On the cut surface the cortex was seen to be narrowed and somewhat spotted, the markings being blurred. The other organs were normal. The central nervous system was not examined.

Histologic examination. The glomerular coils of the *kidneys* displayed large homogeneous masses (Fig. 6) resembling amyloid, in some instances with secondary hyalinization. Similar precipitates were found everywhere in the walls of the small vessels as well as in those of the larger ones, forming thick homogeneous bands of a substance assuming a yellow-brown color when stained according to van Gieson's method. The tubules also contained deposits. Dense plasma cell infiltrates were scattered about and secondary fibrosis had occurred. The amyloid-like substance stained with Jürgens' amyloid stain and with Weigert's fibrin stain, but did not react with Congo red. With Unna's staining method it assumed a deep red color like that of the protoplasm of the plasma cells.

The *spleen* was the seat of diffuse plasma cell infiltration in the pulp. The walls of all vessels contained broad homogeneous bands (Fig. 7) like those of the renal vessels.

The *liver* contained accumulation of plasma cells, partly localized and partly diffusely scattered in the capillaries. The liver cells showed much fatty degeneration, and the vessels contained amyloid deposits which, like those of the other organs, caused a considerable thickening of the walls.

In the *myocardium* the same homogeneous deposits (Fig. 8) were found in the walls of the vessels, but plasma cells were few. There were incipient interstitial fibrosis and some interstitial leukocytic infiltration.

This was therefore a case of paramyloidosis with deposits in the glomeruli and in vessel walls of a number of organs. I have interpreted the alterations in this case as being expressive of a marked positive anergy (plasmacytosis with hyperglobulinosis and atypical amyloidosis). Morphogenetically the paramyloidosis must be considered analogous to the periarterial hyalinosis in case 3 in which there was also an allergic plasmacytosis.

The causal importance of sulfonamides to the paramyloid syndrome in this case (and to the development of allergic hyperglobulinosis in a case later on observed by me) forms a contrast to the well known hypersensitive reactions to sulfonamides. Together they represent reactions of two types which, as described above, form part of the pathology of lupus erythematosus disseminatus.

SUMMARY

Hyperglobulinemia, periarterial fibrosis of the spleen, and the wire loop lesion of the glomeruli in lupus erythematosus disseminatus are all considered to be expressive of a primary allergic hyperglobulinosis in the reticulo-endothelial system, after the analogy of the previously

described (1948) morphologic immunity reaction in atypical and experimental amyloidosis, Boeck's sarcoid, and other conditions.

Periarterial fibrosis of the spleen is thus produced in various conditions with hyperglobulinemia (with or without any demonstrable increase in the number of plasma cells) and displays all transitions to atypical amyloidosis, which, as an underlying cause, also has a stimulation of immune mechanisms with hyperglobulinemia.

Like the periarterial deposits and the collagenic sclerosis (diffuse scleroderma), the characteristic "wire loop lesion" occurring in many cases of lupus erythematosus disseminatus must be looked upon as alterations which are closely related pathogenetically to atypical amyloidosis; whereas focal, and in the narrowest sense allergic, lesions (miliary granulomata in the serosa, nodular necroses, cases of focal allergic pneumonia) are predominant in other cases, or may be present in addition to the lesions first mentioned. From the point of view of immunobiology these two groups of alterations may be considered expressive of a positive anergy and an allergy, respectively.

In some cases also, administration of sulfonamides may give rise to a hyperglobulinotic (paramyloid) syndrome (plasmacytosis, hyperglobulinosis, paramyloidosis in different organs, possibly uremia), in contrast to the well known hypersensitive reactions to sulfonamides.

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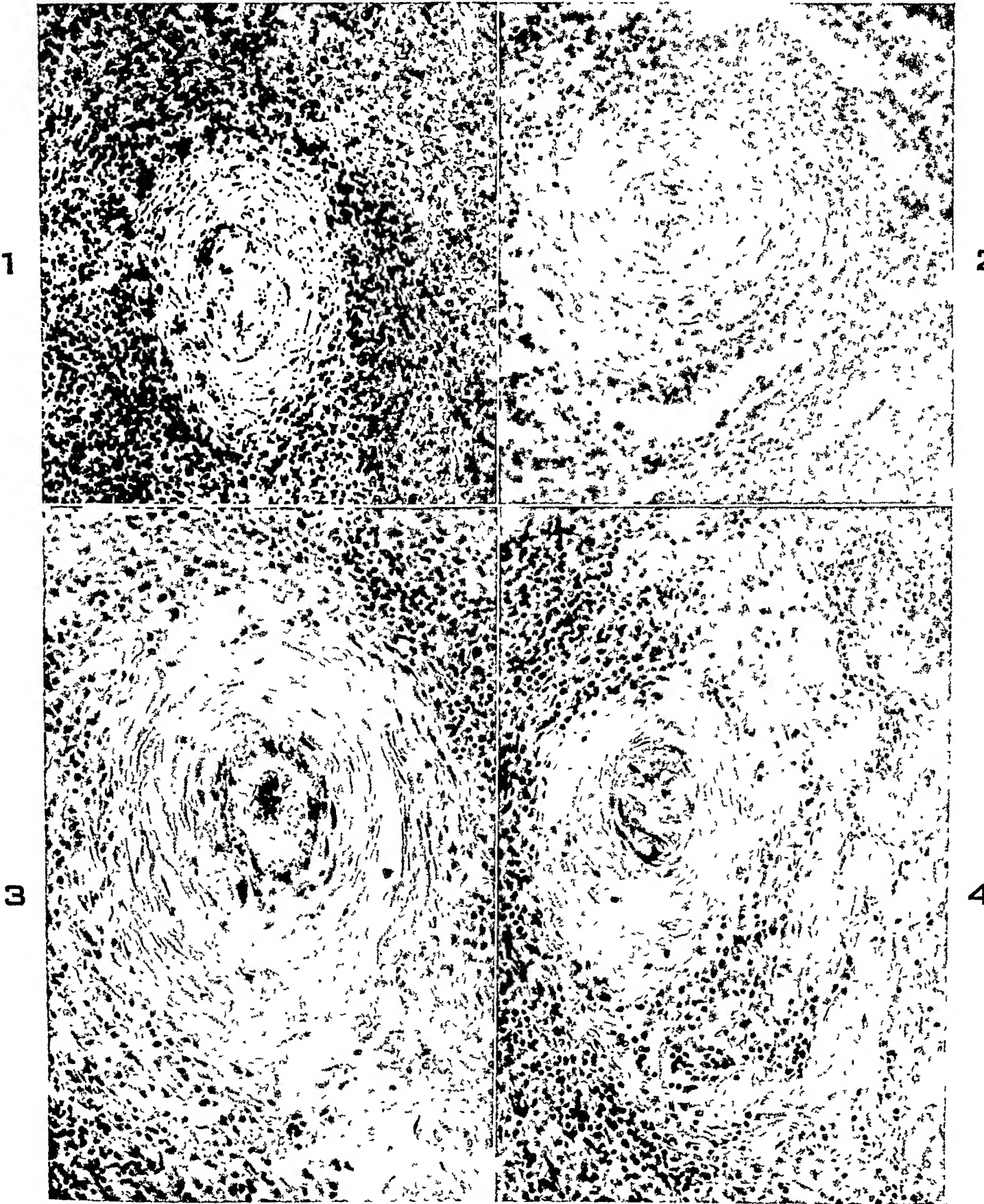
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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 80

- FIG. 1. Case 1. Periarterial fibrosis of the spleen in lupus erythematosus disseminatus. Concentric lamellar hyaline bands. van Gieson-Hansen's stain. $\times 220$.
- FIG. 2. Case 2. Periarterial fibrosis of the spleen in lupus erythematosus disseminatus. Between the rings a few reticulo-endothelial cells are seen. Hematoxylin and eosin stain. $\times 220$.
- FIG. 3. Case 3. Periarterial fibrosis of the spleen in allergic plasmacytosis of the reticulo-endothelial system with marked hyperglobulinemia. Marked accumulation of plasma cells in the border zone. Hematoxylin and eosin stain. $\times 220$.
- FIG. 4. Periarterial fibrosis of the spleen in Boeck's sarcoid with hyalinosi (paramyloidosis). Epithelioid cell granuloma with peripheral paramyloidosis is seen in the border zone. A number of plasma cells and other reticulo-endothelial cells. van Gieson-Hansen's stain. $\times 220$.



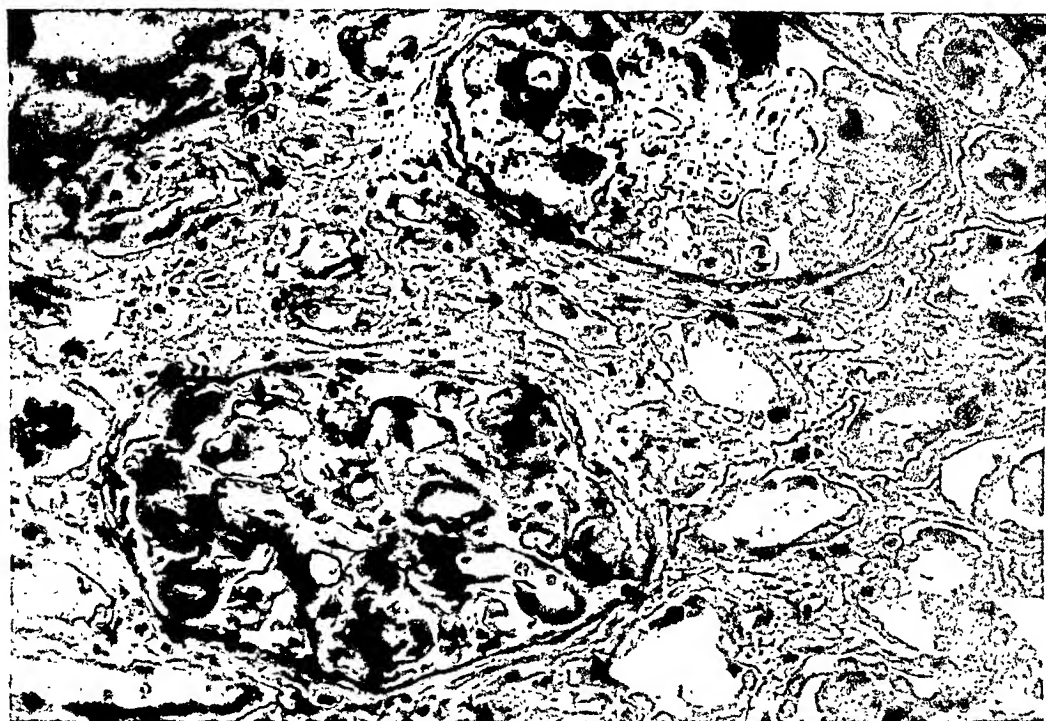
Teilum

Periarterial Fibrosis in Disseminated Lupus

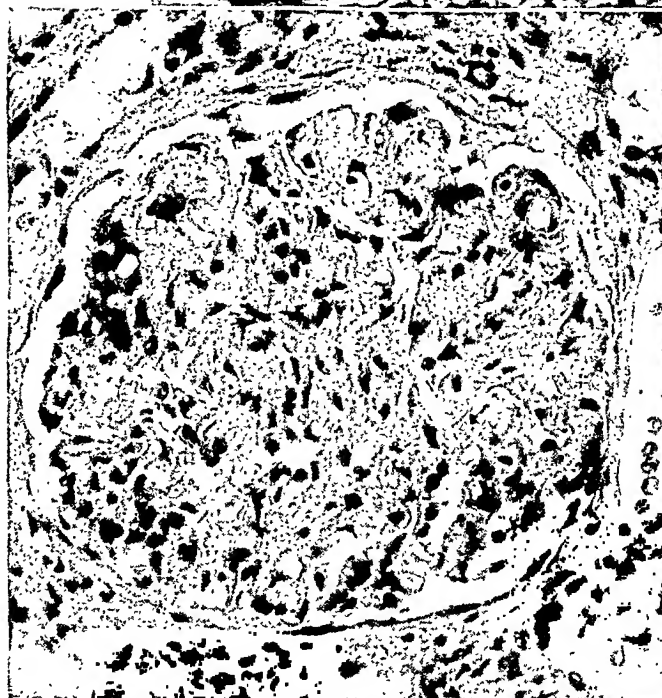
PLATE 81

- FIG. 5. Case 4. Glomerular lesions of chronic polyarthritis with atypical amyloidosis and hyperglobulinemia, for comparison with the wire loop lesion in lupus erythematosus disseminatus. Mallory's stain. $\times 270$.
- FIG. 6. Case 5. Glomerular lesions in allergic plasmacytosis with paramyloidosis and hyperglobulinemia, for comparison with the wire loop lesion in lupus erythematosus disseminatus. Hematoxylin and eosin stain. $\times 310$.
- FIG. 7. Case 5. Paramyloid deposits in the splenic vessels. van Gieson-Hansen's stain. $\times 220$.
- FIG. 8. Case 5. Paramyloid deposits in the vascular walls of the myocardium. Hematoxylin and eosin stain. $\times 120$.

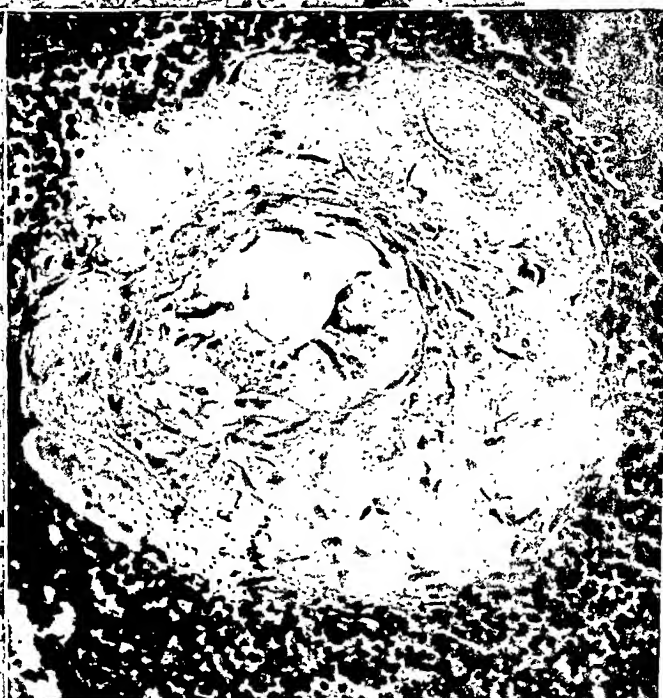
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Teilum

Periarterial Fibrosis in Disseminated Lupus

EFFECTS OF ALLOXAN UPON FUNCTION AND STRUCTURE OF NORMAL AND NEOPLASTIC PANCREATIC ISLET CELLS IN MAN *

JEROME W. CONN, M.D., AND DORIN L. HENNERMAN, M.D.

(From the Departments of Internal Medicine and Pathology, University of Michigan, Ann Arbor, Mich.)

Since the original demonstration in 1943 by Dunn, Sheehan, and McLetchie¹ that alloxan administered to rabbits produces selective necrosis of the pancreatic islets of Langerhans, it has been established that a single injection of this substance, varying from 50 to 400 mg. per kg. of body weight, is capable of producing destructive changes in the normal islets of the pigeon,² duck,³ rat,^{4,5} cat,⁶ dog,⁶ and monkey.^{4,7} It has been found, too, that a series of smaller daily doses of alloxan produces islet cell degeneration but that the total dose required is considerably greater.^{8,9} Under these circumstances the degenerative changes in the islet cells are less acute and evidence of attempted repair of damaged islets is observed in the rabbit.^{8,10,11} Regeneration of damaged islets has been observed in the rabbit following a single small dose of alloxan.^{12,13}

Published observations on the effects of alloxan upon the pancreas of man are limited to the 2 cases reported by Brunschwig *et al.*^{14,15} In one case the pancreas was examined 7 hours after a single intravenous injection of 600 mg. per kg. of alloxan. In this case "microscopic study of the pancreas revealed questionable evidence of injury to a number of cells in some of the islets, although many islets were not affected." In the other, a case of islet cell carcinoma with metastases, no microscopic evidence of injury either of the normal islets or of the tumor cells was observed. This pancreas had been examined 1 month after the last injection of alloxan. In the preceding 50-day period the drug had been given in large doses (total 3,150 mg. per kg.) irregularly. Thus, in the 2 cases studied heretofore, no clear evidence of a destructive effect of alloxan upon human islet tissue has been observed. The impression has been gained, therefore, that the pancreatic islet cells of man are exceedingly resistant to the damaging effects of alloxan. That this conclusion is not warranted on the basis of so few observations is indicated by the fact that in our studies the sensitivity of human islet tissue to the destructive effects of alloxan approximates that observed for other species.¹⁶ In addition, we found that neoplas-

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tic islet cells are much more resistant, both anatomically and functionally, to the damaging effects of alloxan than are normal islet cells.

Our observations were made upon a patient who satisfied all the criteria needed for a diagnosis of organic hyperinsulinism.¹⁷ Subsequently, an islet cell tumor was shown to have been the cause of the hyperinsulinism. A brief summary of the history and clinical course of this patient follows.

REPORT OF CASE

A.W., a white housewife, 58 years old, was admitted to the University Hospital on May 14, 1946, complaining of attacks of unconsciousness. Ten years previously she had noted the onset of severe early morning fatigue which was relieved by eating breakfast. This symptom gradually progressed in severity. Four years prior to admission pre-breakfast attacks appeared which were characterized by memory defects, blurring of vision, clouding of consciousness and, at times, inability to awaken in the morning. The great majority of such episodes occurred between 5 a.m. and 8 a.m. but occasionally one was experienced in the forenoon. The frequency and severity of the attacks continued to increase. During the year before admission attacks had occurred almost daily. In an effort to prevent attacks by eating frequently, the patient had gained 36 pounds during the preceding 3 years.

Except for obesity, the physical examination revealed no abnormalities. Blood pressure was 130/70 mm. of Hg.

The Kahn reaction of the blood was positive. Spinal fluid gave a negative Kahn reaction. Except for the abnormally low levels of the blood sugar outlined below, all other laboratory examinations showed no abnormality. These included blood counts, urine analyses, tests of kidney and liver function, x-rays of the skull and chest, and determinations of blood nonprotein nitrogen, basal metabolic rates, serum proteins, and plasma cholesterol.

PROCEDURES AND RESULTS

Summary of Metabolic Data

From May 14, 1946, to November 25, 1946 (193 days), extensive metabolic data were obtained and will be reported in detail elsewhere.¹⁸ For the purpose of integrating the functional and histologic changes observed, the major metabolic findings before, during, and after the administration of alloxan are outlined.

Pre-alloxan Period (35 Days)

Daily fasting blood sugar ranged from 15 to 62 mg. per cent with an average level of 31 mg. per cent. Repeated oral and intravenous glucose tolerance tests, performed after standard dietary preparation,^{19,20} demonstrated a greatly increased tolerance for carbohydrate. One meal low in carbohydrate (17 gm.) resulted shortly in intense hypoglycemia requiring glucose intravenously.

Alloxan Period (9 Days)

Alloxan was administered intravenously on 9 consecutive days in the following amounts: 50, 50, 100, 100, 100, 100, 100, 100, and 100 mg.

per kg., for a total of 800 mg. per kg. The patient weighed 75 kg. and thus received 60 gm. of alloxan during the 9-day period. The diet was constant. The daily fasting blood sugar ranged from 29 to 54 mg. per cent with an average level of 40 mg. per cent. No untoward reactions occurred. Daily examination of the urine showed no albumin or cellular elements. For several hours after the administration of alloxan the urine was light red. No hemoglobin was present. The color was due to the excretion of a reduction product of alloxan. The blood nonprotein nitrogen and daily white blood cell count remained normal as did the bromsulfalein test for liver function.

Post-alloxan Period

Before Operation (15 Days). The daily fasting blood sugar ranged from 34 to 60 mg. per cent with an average level of 45 mg. per cent. For the last 4 days of this period the levels were 41, 34, 45, and 35 mg. per cent, respectively. Repeated glucose tolerance tests done in this period indicated that despite the continuation of an abnormally low level of the fasting blood sugar, a marked decrease in carbohydrate tolerance had occurred as a result of alloxan administration. The persistence, however, of severe pre-breakfast hypoglycemic attacks made surgical exploration necessary.

Operation. On July 12, 1946, surgical removal of an islet cell tumor of the pancreas was performed by Dr. Robert W. Buxton, of the University Hospital staff. The tumor measured 1.5 by 1.0 by 1.0 cm., and was located in the body of the pancreas. Specimens of the pancreas proper and of the liver were removed for microscopic study.

After Operation (134 Days). Within 20 hours following removal of the islet cell tumor frank diabetes had developed with heavy glycosuria, ketonuria, and a fasting blood sugar of 208 mg. per cent. Insulin therapy was begun on the second postoperative day. Ketonuria disappeared in 2 days. Despite the use of 20 to 35 units of insulin daily (average, 30 units) and a low carbohydrate intake, the daily fasting blood sugar for the first 11 postoperative days ranged from 146 to 234 mg. per cent with an average level of 186 mg. per cent. An increase of insulin to as high as 60 units per day for several days brought the fasting blood sugar to normal where it remained for the next 16 days on a dose of 20 to 30 units per day. Insulin was gradually discontinued and the fasting blood sugar remained normal. However, postprandial glycosuria still occurred. Repeated glucose tolerance tests continued to show consistently normal fasting blood sugar levels, but they also demonstrated the hyperglycemic plateau type of curve typical of diabetes mellitus. From the character of serial curves it appears likely that normal tolerance for carbohydrate will eventually return.

Structural Changes

Methods of Preparation of Tissues. The tumor was bisected, photographed and the halves were immediately placed in Bouin's fixative and in 10 per cent formalin. Small pieces of pancreas proper and of liver were placed in similar fixatives.

The following stains were used in the study of the tissues:

Hematoxylin and eosin stain	Gomori's chromium hematoxylin-phloxine stain
Mallory-Heidenhain azan stain	Van Gieson's stain
Mallory's phosphotungstic acid hematoxylin stain	Bensley's acid fuchsin and methyl green stain
Bensley's modification of Mallory's aniline blue stain	Warthin-Starry-Kerr silver stain
Ziehl-Neelsen's stain	

Evaluation of Staining Procedures Utilized. The general histologic features were brought out nicely by the hematoxylin and eosin stain, Gomori's chromium hematoxylin-phloxine stain, Mallory's phosphotungstic acid hematoxylin stain, and the Mallory-Heidenhain azan stain.

The use of many sections of normal and diseased pancreatic tissue as controls proved that Gomori's^{21,22} stain distinguished beta cells from those of the alpha and delta types more efficiently than other stains utilized. By this technic the normal beta cells possessed a very fine, dust-like, blue granular cytoplasm whereas the alpha and delta cells were indistinguishable from each other and had a pink to red, more coarsely granular cytoplasm.

The identity of alpha cells was confirmed by Mallory's phosphotungstic acid hematoxylin stain.²² The alpha granules took a dark blue stain. These cells corresponded to the alpha cells by the Gomori stain. The Mallory technic (phosphotungstic acid hematoxylin) differentiated alpha from delta cells since the latter were not stained by this method.²²

The Mallory-Heidenhain azan stain was useful but alpha and delta cells failed to stain as brilliantly as reported by Gomori.²² Beta cells stained a pale gray, while delta cells were blue.

Bensley's procedures were not done on fresh material, but those recommended for fixed material^{23,24} were uniformly unsatisfactory in our hands.

The Warthin-Starry-Kerr method²⁵ and Ziehl-Neelsen's stains were negative for organisms. Van Gieson's stain was used for study of the hyaline material, which took a pale yellow to pale pink color.

Gomori's chromium hematoxylin-phloxine stain was used with slight modifications in timing. The hematoxylin solution was allowed to age for at least 1 week instead of 48 hours as recommended. Sections were exposed to both staining solutions about twice as long as Gomori²² suggested and differentiation and decolorization procedures were corre-

spondingly increased. Greater contrast and better detail were obtained by this longer procedure.

Islet Cell Counts

The islets of the pancreas proper were segregated according to the degree of damage which they exhibited and the component cells were studied and tabulated according to qualitative and quantitative changes. Inasmuch as the cells of the adenoma failed to show the qualitative and quantitative changes characteristic of alloxan injury, these cells were not counted individually but an approximation was made of the frequency of cell types on the basis of the granule stains.

Gross Features of the Islet Cell Neoplasm

In situ the tumor presented as a slight protrusion above the body of the pancreas. The neoplasm was slightly firmer than the surrounding pancreas. A rich vascular network coursed over its surface, a feature which aided in distinguishing the tumor from enlarged peripancreatic lymph nodes.

Upon removal and sectioning, the tumor was found to be coarsely lobulated. It weighed about 1.5 gm., a weight which is equivalent to the estimated combined weight of all of the islets in the average normal pancreas.²⁶ It measured 1.5 by 1.0 by 1.0 cm. Most of the periphery was well encapsulated and well demarcated from adjacent pancreatic tissue. However, localized peripheral areas showed no distinct capsule. The cut surface was primarily a dull gray with irregular blotches of dull reddish purple.

Histopathologic Features of the Islet Cell Tumor

With regard to general architecture and cellular type, the tumor removed from this patient conformed in all respects to the numerous excellent descriptions which are readily available in the literature.²⁷⁻³³ Suffice it to say that the pattern of cells and their structure were almost identical with those observed in normal or in hyperplastic islets of Langerhans. The following description is aimed at emphasizing (1) the possible genesis of the neoplasm, (2) age differences in various portions of the tumor, (3) cell types according to specific granule stains, and (4) the complete absence within the tumor of evidence of alloxan injury.

In the areas where the fibrous connective tissue capsule was lacking, multiple small nodules representing centers of active proliferation were found. Many of these proliferating nodules had surrounded and compressed acinar tissue, islets, and fat lobules. Many ducts and ductules were seen in intimate relation with the tumor cells. Ductules possess-

ing terminal bars and relatively heavy basement membranes were easily identified in these nodules. Commonly these ductules were seen to end in the tumor. The epithelium of the ductules blended with and appeared to give rise to the cells of the neoplasm. At least 70 per cent of the cells in some of these small nodules appeared to be the epithelial cells of ductules. They were indistinguishable from such epithelial cells in size, shape, cytoplasmic granules, and staining reactions. These cells were larger than normal islet cells but were somewhat smaller than most of the cells of the neoplasm. They also possessed a darker staining cytoplasm than most of the cells of the tumor. They were columnar or polyhedral. Cytoplasmic granules were coarser than those of beta cells. These granules showed no particular affinity for either of the specific colors in the granule stains. Scattered among these undifferentiated cells were a significant number of cells showing transformation to a beta-like cell in that a varying degree of development of granules of the beta type were seen within them. Mitotic figures could not be found in these or other portions of the tumor. On the basis of these findings, it was considered that the neoplastic islet cells seen in this tumor had their origin in duct epithelium.

The larger and older nodules of the tumor showed few ducts and little evidence of proliferation or of transformation of duct epithelium to tumor cells. Dense hyaline tissue was considerably more abundant in these areas.

Eighty-five to 90 per cent of the cells resembled beta cells in their staining reactions. Approximately 10 per cent of these possessed their full complement of granules. The remaining cells of the beta type demonstrated varying degrees of degranulation. Less than 1 per cent of the cells contained granules stainable like those of alpha cells, either by Gomori's method or by Mallory's phosphotungstic acid hematoxylin stain. The rest of the cells were either degenerating tumor cells showing hyalinization or they were undifferentiated cells which could not be classified. Surrounding the tumor and in the interstices of the pancreas were foci of heavy inflammatory reaction.

The most striking feature of the cells of this tumor was the complete absence of destructive changes which could be attributed to alloxan injury. In marked contrast were the intense degenerative changes, characteristic of alloxan injury, which were observed in the islets of the pancreas proper.

Histopathologic Features of the Pancreatic Islets

Virtually every islet in the pancreas showed some degree of damage attributable to alloxan injury. The degree of change varied greatly

from islet to islet. When examined after staining with routine hematoxylin and eosin the extent of the damage seemed slight. Islets were small and some of the cells showed vacuolar degeneration. However, the specific granule stains revealed profound changes both qualitatively and quantitatively.

The qualitative changes, which predominantly involved the beta cells, were many and varied. Minor degrees of alteration (some of which were probably not the result of alloxan *per se*) included degranulation, marked cloudy swelling, vacuolar degeneration, and other cytoplasmic disruptions. More severe changes involved loss of cytoplasm and cell membranes, pyknosis and fragmentation of nuclei proceeding to necrosis and disappearance of cells. With disruption of cell membranes and cytoplasmic boundaries there was collapse of entire islets. If severe, this process resulted in small shrunken masses of cells without the usual complexity of architecture seen in normal islets. Entire islets were destroyed leaving only a few shadows of former islet cells, or a few disorganized cells among cellular débris. However, the amount of cellular débris was small in comparison with the severe destruction observed, indicating rapid removal of the destroyed cells.

Normally the average island of Langerhans contains at least as many or more beta cells than alpha and delta cells combined²¹ and evidence of degeneration is lacking. The changes which we observed in these islets included the complete disruption of the normal ratio of alpha to beta cells. Careful cell counts of 128 islets showed that 82 per cent were alpha or delta cells. Only 18 per cent were beta cells. Of these, 89 per cent showed significant degenerative changes. Thus, less than 2 per cent of all of the cells studied in the islets could be considered to be normal beta cells. With a normal expectancy of at least 50 per cent normal beta cells, this represented a degeneration and/or disappearance of 96 per cent of all of the beta cells. For purposes of classification and of comparative cell counting, the islets were divided into five groups:

1. "Least injured" (approximately normal size and architecture).
2. "Collapsed" (moderate loss of cells and disruption of normal pattern).
3. "Shrunken" (severe loss of cells and disruption of normal pattern).
4. "Completely destroyed" (remnants and débris indicating the site of a former islet).
5. "Hyperplastic" (enlarged, increased number of cells and exaggerated ribbon pattern).

1. As will be noted in Table I, 42 islets (33 per cent) were "least

injured." In only 4 of these did the ratio of alpha + delta to beta cells fall within the normal range. In these 4 islets 53 per cent of cells were beta cells. But 52 per cent of these beta cells demonstrated significant destructive changes, such as marked cloudy swelling, vacuolar change, pyknosis, and necrosis. Forty-seven per cent of the cells belonged to the alpha-delta series and appeared to be unharmed.

TABLE I

Qualitative and Quantitative Analysis of All Types of Islet Cells Encountered in 128 Islets of Langerhans (15 Days after the Administration of Alloxan)

Classification of 128 islets studied	Number of islets in each group	Disappearance of beta cells, average percentile decrease per islet*	Condition of remaining beta cells		Condition of alpha and delta cells
			Normal	Degenerative changes	
Least injured	42 (33%)	4 islets 0%	48%	52%	Normal
		38 islets 22%	23%	77%	Mild degenerative changes
Collapsed	27 (21%)	72%	7%	93%	Mild degenerative changes
Shrunk	33 (26%)	88%	0	100%	Moderate degenerative changes
Completely destroyed (remnants and debris)	19 (15%)	100%			Disappearance of recognizable cells
Hyperplastic	7 (5%)	80%	26%	74%	Marked hyperplasia with brilliant staining (newly formed cells)

* Based upon the maximal normal ratio, $\frac{\text{alpha} + \text{delta cells}}{\text{beta cells}} = 1.0$.

The other 38 islets in this "least injured" group revealed a decrease in beta cells with a greater number showing destructive changes. In this group there were 39 per cent beta cells of which 77 per cent showed destructive changes. On the basis of the least expected number of beta cells in an unchanged islet there had been complete destruction and disappearance of at least 22 per cent of the beta cells. Sixty-one per cent of the cells were of the alpha-delta series. The granules of these alpha cells revealed a somewhat reduced affinity for the specific stains and occasionally they showed moderate cloudy swelling.

2. Islets showing varying degrees of "collapse" composed 21 per cent of the total islets counted. Only 14 per cent of the cells were beta cells, a figure which is much reduced from the 39 per cent seen in the previous group of islets. At least 72 per cent of the beta cells had been

completely destroyed. Of the relatively few beta cells remaining, a greater number (93 per cent) demonstrated significant degenerative changes. Many cells of the alpha-delta series in this group of islets also showed mild degenerative changes.

3. Twenty-six per cent of the total number of islets showed severe destructive changes resulting in "shrunk" islets. Of the cells present, only 6 per cent were beta cells, representing a complete disappearance of at least 88 per cent of beta cells. Ninety-three per cent of the cells of the islets were alpha and delta cells. The staining of the cytoplasmic granules in these alpha-delta cells was definitely impaired as compared with normal alpha and delta cells. In the severely shrunk islets many of the pink-staining cells were small spindle-shaped structures with little cytoplasm.

4. Fifteen per cent of the islets were completely destroyed. Perhaps many more belonged in this category, but sufficient proof of former islet architecture was lacking. Only a few disrupted islet cells or their "ghosts" were present. Sometimes a few spindle-shaped pink-staining cells and numerous ductules were observed. Nearly all of the cells seen stained pink and contained no definite granules.

5. Five per cent of the islets demonstrated the typical exaggerated ribbon pattern of hyperplastic islets. Strikingly, about 90 per cent of the cells in these islets were alpha and delta cells; and, of these, an unusual number were delta cells as shown by Mallory's phosphotungstic acid hematoxylin method. By this method and by other specific granule stains, the alpha and delta granules of the cells of these hyperplastic islets took a very brilliant stain suggesting that these cells were not present during the destructive insult which decreased the stainability of the older alpha and delta cells, and that this may represent regenerative proliferation of alpha and delta cells. The presence of an increased number of ducts within and surrounding the hyperplastic islets supports this opinion. Seventy-four per cent of the few beta cells which were present (10 per cent of the total in the hyperplastic islets) revealed degenerative changes.

Liver Obtained for Biopsy

The liver cells were well filled with glycogen. They showed no evidence of injury. The liver lobules were intact and appeared to be normal. There was, however, an increase in young connective tissue in the portal trinites with an accompanying slight inflammatory infiltration of lymphocytes and mononuclear cells. Several tubercle-like foci of epithelioid cells were encountered. They bore a very close resemblance to those observed by Dunn and co-workers³⁴ in the

regional lymphoid tissue of a rabbit after the intravenous administration of alloxan.

DISCUSSION

There can be no doubt that the severe degenerative changes that we observed in the pancreatic islets of this patient were the direct result of the alloxan which had been administered. The changes are typical of those observed in the islets of alloxan-treated animals. They resemble most closely those described by Bailey *et al.*⁸ as occurring in rabbits given small daily injections of alloxan for from 7 to 13 days.

The intensity of the alloxan-induced damage to the beta cells of the islets is indicated by the facts that (1) 64 per cent of all the beta cells had disappeared, (2) of the beta cells remaining, 90 per cent showed degenerative changes, and (3) only 2 per cent of all of the cells seen in the islets could be regarded histologically as normal beta cells. This degree of structural damage to the pancreatic islets of a human being resulted from the administration of a total of 60 gm. of alloxan (800 mg. per kg. of body weight) given intravenously in divided doses over a 9-day period. The amount of alloxan (on a per kg. basis) required to produce these changes falls close to that needed to produce similar islet cell damage in other species when the drug is given in daily divided doses.^{8,9} One is forced to conclude, therefore, that in this study the sensitivity of human pancreatic islet tissue to the destructive effects of alloxan approximates that observed for other species. This is in sharp contrast to the statement found repeatedly in the literature to the effect that the pancreatic islet tissue of man is resistant to the damaging effects of alloxan. This conclusion is based upon the observations of Brunschwig *et al.*^{14,15} in 2 cases in which the pancreas was examined after the administration of alloxan. While there is no ready explanation for the discrepancy between our findings and theirs, one may suggest several possibilities.

It is recognized that an occasional animal of any species is found to be resistant to the destructive effects of alloxan.^{6,35,36} It is conceivable that Brunschwig's first patient,¹⁵ who had received tremendous amounts of alloxan, fell into such a category. The other patient,¹⁵ having received a single dose of alloxan (600 mg. per kg. intravenously), died 7 hours later. Questionable histologic evidence of injury was reported. It is possible that more positive evidence of islet cell damage would have been observed had the patient survived for a longer period of time. In this connection, it is of interest that we, too, have observed early degenerative changes in the pancreatic islets of a patient who died 9 hours after a single intravenous dose of alloxan amounting to 215 mg. per kg.³⁷ This patient had been suffering from

long-standing organic hyperinsulinism, shown at autopsy to have been due to marked generalized hyperplasia of the islets of Langerhans. He was moribund upon admission to the hospital and death could not be attributed to the alloxan which had been administered.

Thus it appears premature to conclude that human pancreatic islet tissue is less susceptible to damage from alloxan than is islet tissue of every other species studied. This point is of considerable importance with regard to present and future investigations upon the possible rôle of endogenous alloxan or related compounds in the etiology of some types of diabetes mellitus in man. The "resistance" to alloxan of human islet cells has been a potent argument against such speculations.

Of both practical and theoretical interest is the fact that human *neoplastic* islet tissue is very much more resistant to the destructive effects of alloxan than is normal islet tissue. In this study the normal islet cells have served as a control for the neoplastic ones. Both were exposed to the same insult. While very few normal beta cells remained in the islets of Langerhans, the beta-like cells of the tumor showed no evidence of alloxan injury. Since it has been reasonably well established that alloxan exerts its injurious effect upon cellular metabolism, by virtue of its capacity to oxidize sulfhydryl groups, thus interfering with certain essential intracellular enzymatic oxidation-reduction systems,³⁸ it would appear that the metabolism of neoplastic islet tissue differs in important respects from that of normal islet cells. It is suggested, further, that the hypoglycemia-invoking product secreted by neoplastic islet cells may not be identical with the crystalline protein substance obtainable from normal islet tissue.

From the practical point of view it would appear that attempts to degenerate islet cell neoplasms by means of the administration of alloxan are fraught with danger. It must be realized first that were the attempt successful it almost certainly would be associated with complete degeneration of all of the normal insulin-producing islet tissue in the body. It is more than likely, however, that the amount of alloxan required (if it affected neoplastic islet tissue at all) would be lethal to the patient, since the compound is injurious to tissues generally.

The metabolic studies made upon this patient will be reported in detail elsewhere.¹⁸ They confirm, from a functional point of view, all of the conclusions which can be drawn from study of the structural alterations described. Following the administration of alloxan there occurred a remarkable decrease in the patient's ability to metabolize ingested carbohydrate. The degree of impaired carbohydrate tolerance was comparable to that seen in diabetes mellitus. The striking point

of difference in this patient, however, was the persistence of very low levels of daily fasting blood sugar (pre-breakfast level). On the basis of these findings it was concluded clinically that the islet cell tumor (which is responsible for the pre-breakfast hypoglycemia always observed in these patients)¹⁷ had been but little, or not at all, affected by alloxan. It was reasoned that the sharp decrease which had occurred in the patient's tolerance for carbohydrate must have been due to injury of normal islet tissue. These conclusions seemed warranted in the presence of continued normal hepatic function. The prompt development of typical and persistent diabetes mellitus following surgical removal of the islet cell tumor completed the clinical story and related it in all of its details to the anatomic observations described.

Several other observations from the structural point of view seem worthy of comment. An occasional islet cell in the pancreas proper was observed to be undergoing hydropic degeneration. This is the type of islet cell degeneration which has been associated experimentally with procedures known to place excessive functional demands upon the islets,^{39,40} a form of "overwork degeneration." This change is not usually found in animals as the result of acute alloxan injury, although it has occasionally been observed.⁸ It seems likely that this change when it occurs is not a direct but an indirect effect of alloxan. Those beta cells which have escaped the damaging effects of alloxan are now subject to excessive functional strain and may undergo "overwork degeneration."

Although the functions of the alpha and delta cells of the islets of Langerhans are not known, two types of change involving these cells were observed in our sections. It was noted (Table I) that in those islets that suffered the greatest degree of beta cell injury, the alpha and delta cells showed mild to moderate decrease in stain-affinity, suggesting a slight degree of injury. The other change was related to the presence of occasional hyperplastic islets. Ninety per cent of the cells of these islets were alpha and delta cells. The unusually brilliant staining of these cells and the increased numbers of ducts surrounding these hyperplastic islets suggest that the large islets represent a proliferative response of alpha and delta cells which followed the alloxan injury.

Finally, sections of the beta-cell-like tumor indicate strongly that it arose from duct epithelium. The question of the origin of islet cells from duct epithelium has been a controversial one since Bensley²³ and Grauer⁴¹ first suggested it. Direct continuity between duct epithelium and cells of islet tumors has been observed repeatedly.^{28,29,42-44} These observations have led Laidlaw²⁸ and O'Leary and Womack²⁹ to postulate that islet cell tumors originate in response to a stimulus to duct

epithelium, and that this particular stimulus calls forth the islet cell potentiality of duct epithelium. There is much to support the opinion that the neoplasm in our case, secreting an insulin-like substance, arose from and continued to receive from duct epithelium, undifferentiated cells which matured to become beta-cell-like tumor cells.

CONCLUSIONS

1. In this study the sensitivity of normal human islet tissue to the destructive effects of alloxan was found to approximate that observed in other species.

2. Neoplastic islet tissue is much more resistant to the injurious effects of alloxan than is normal islet tissue. This suggests that certain metabolic processes within the abnormal cells differ significantly from those which exist in normal islet cells.

3. The development of persistent diabetes mellitus following surgical removal of the uninjured islet cell neoplasm confirms functionally (a) the severe structural lesion observed in the islets of the pancreas proper and (b) the absence of structural alterations in the tumor cells.

4. In future attempts to use alloxan therapeutically the above facts should be considered. It is believed that the amount of alloxan required to produce the desired effect upon islet cell neoplasms is likely to be lethal to the patient.

5. Alpha and delta cells of human islet tissue are susceptible to damage by alloxan, but to a much lesser degree than are beta cells.

6. Hyperplasia of alpha and delta cells appears to be one response to alloxan injury of the islets of Langerhans.

7. In this case the evidence is good that the islet cell tumor had its origin in pancreatic duct epithelium.

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[Illustrations follow]

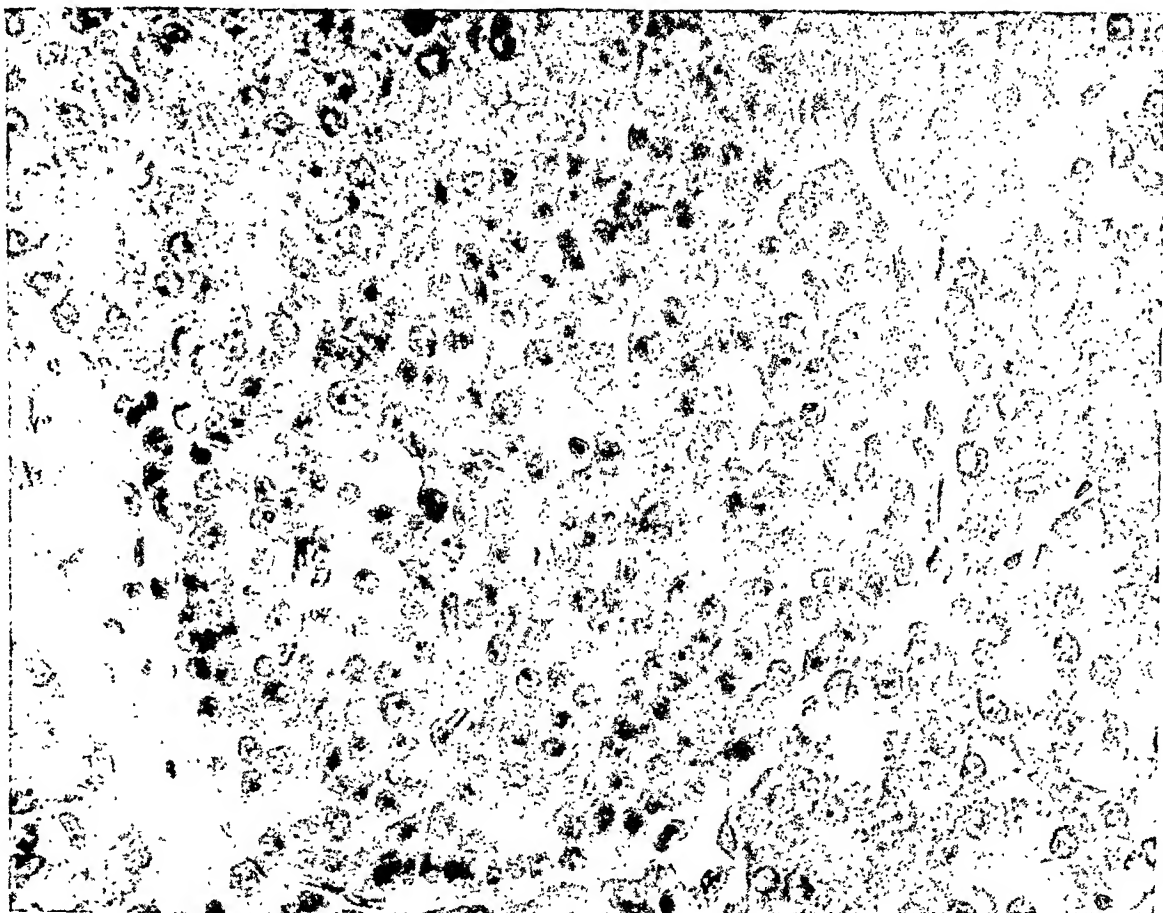
DESCRIPTION OF PLATES

PLATE 82

FIG. 1. Relatively normal or "least injured" islet of Langerhans showing normal size, structure, and ratio of alpha+delta to beta cells. The cells with dark cytoplasm are normal beta cells. Scattered beta cells show significant degenerative changes and actual necrosis. Gomori's chromium hematoxylin-phloxine stain. $\times 125$.

FIG. 2. A moderately collapsed islet with smaller size and somewhat altered structure composed largely of alpha and delta cells. Small masses of cellular debris represent destroyed beta cells. A large number of remaining beta cells show severe degenerative changes as described. Gomori's chromium hematoxylin-phloxine stain. $\times 125$.

1



2

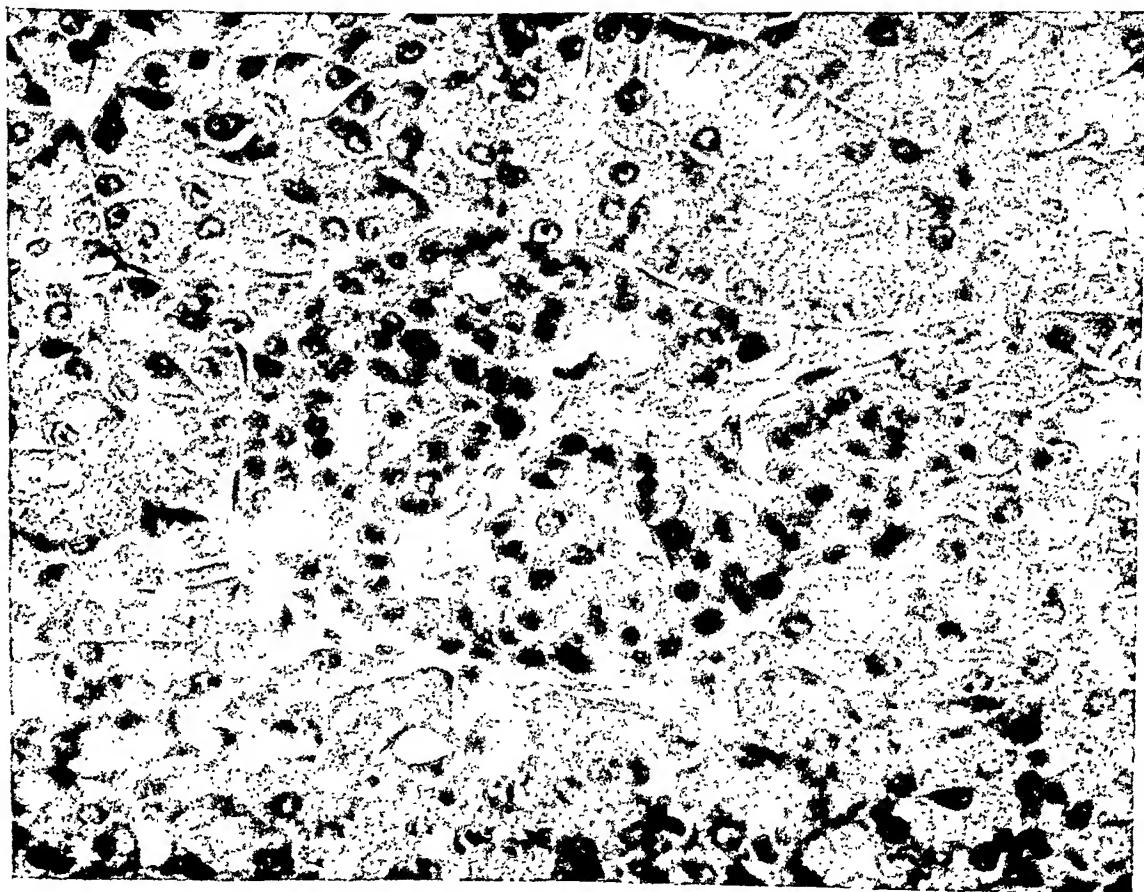
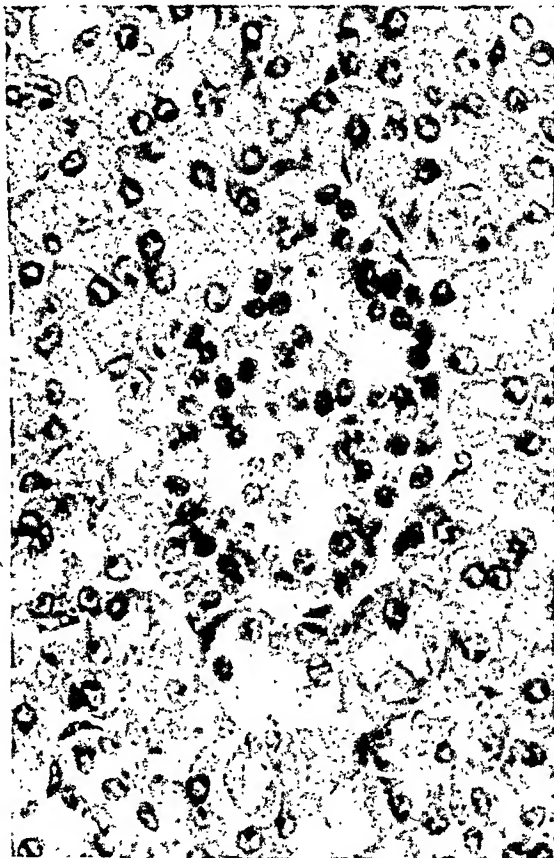


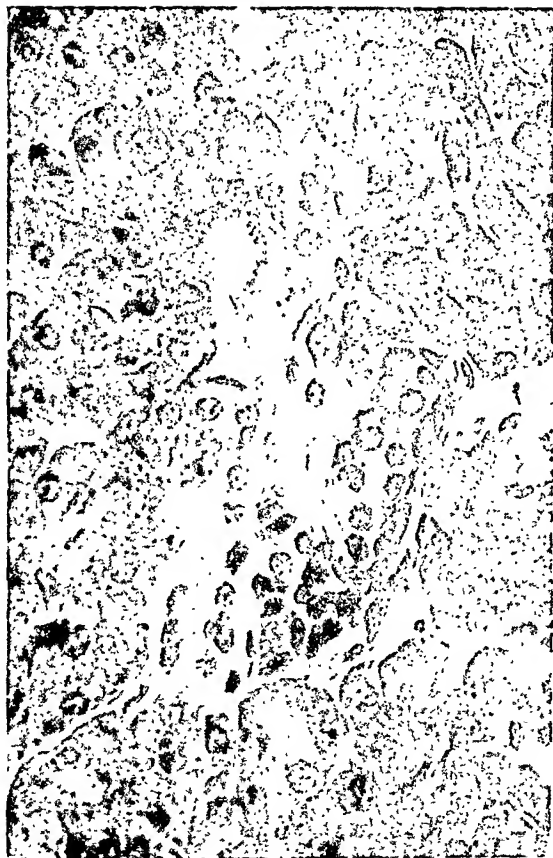
PLATE S3

- FIG. 3. Small islet showing a more severe degree of collapse. The centrally arranged beta cells are largely necrotic. The peripherally arranged alpha and delta cells show slight degenerative changes and impaired stainability of granules. There is shrinkage of the entire islet structure. Gomori's chromium hematoxylin-phloxine stain. $\times 125$.
- FIG. 4. A greatly shrunken islet composed largely of a reduced number of spindle cells possessing a pale pink cytoplasm. No normal beta cells are found. Gomori's chromium hematoxylin-phloxine stain. $\times 125$.
- FIG. 5. A completely destroyed islet showing only connective tissue hyalin outlining the former islet pattern, and a few isolated bizarre cells. Gomori's chromium hematoxylin-phloxine stain. $\times 125$.

3



4



5

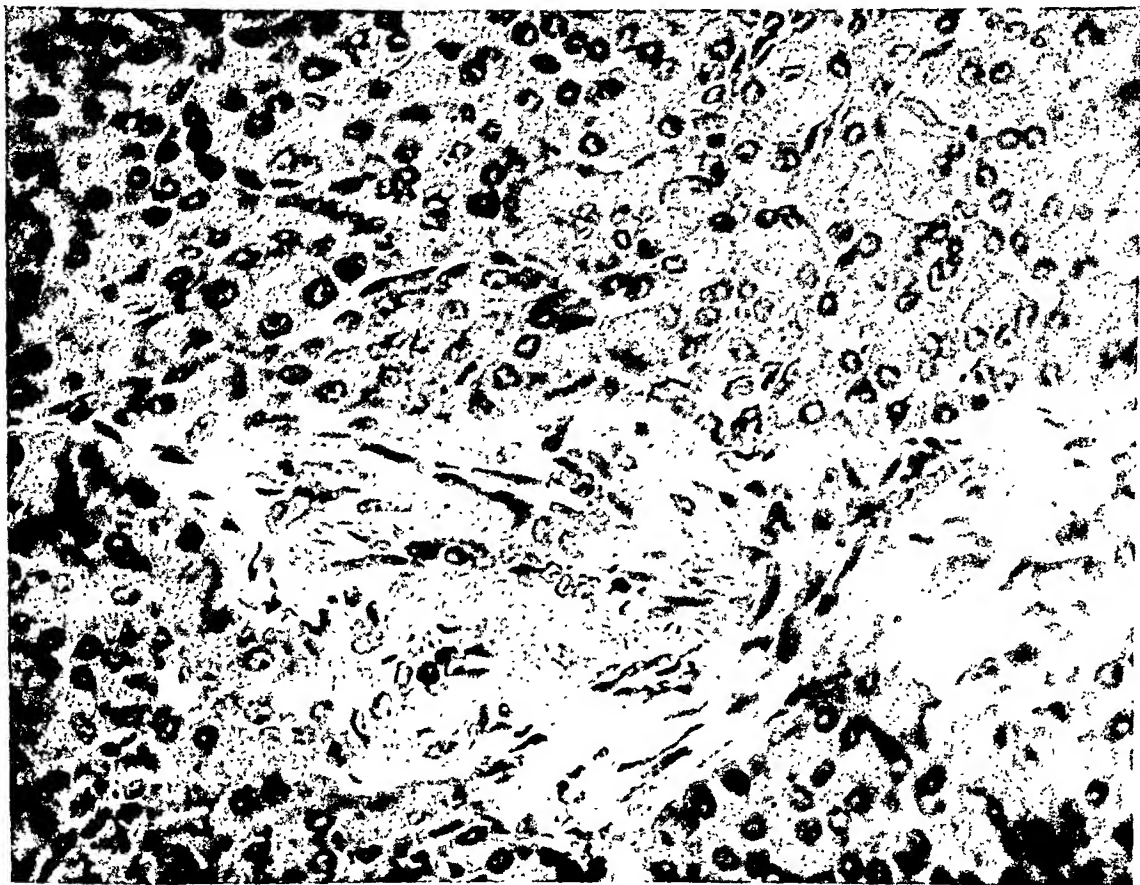
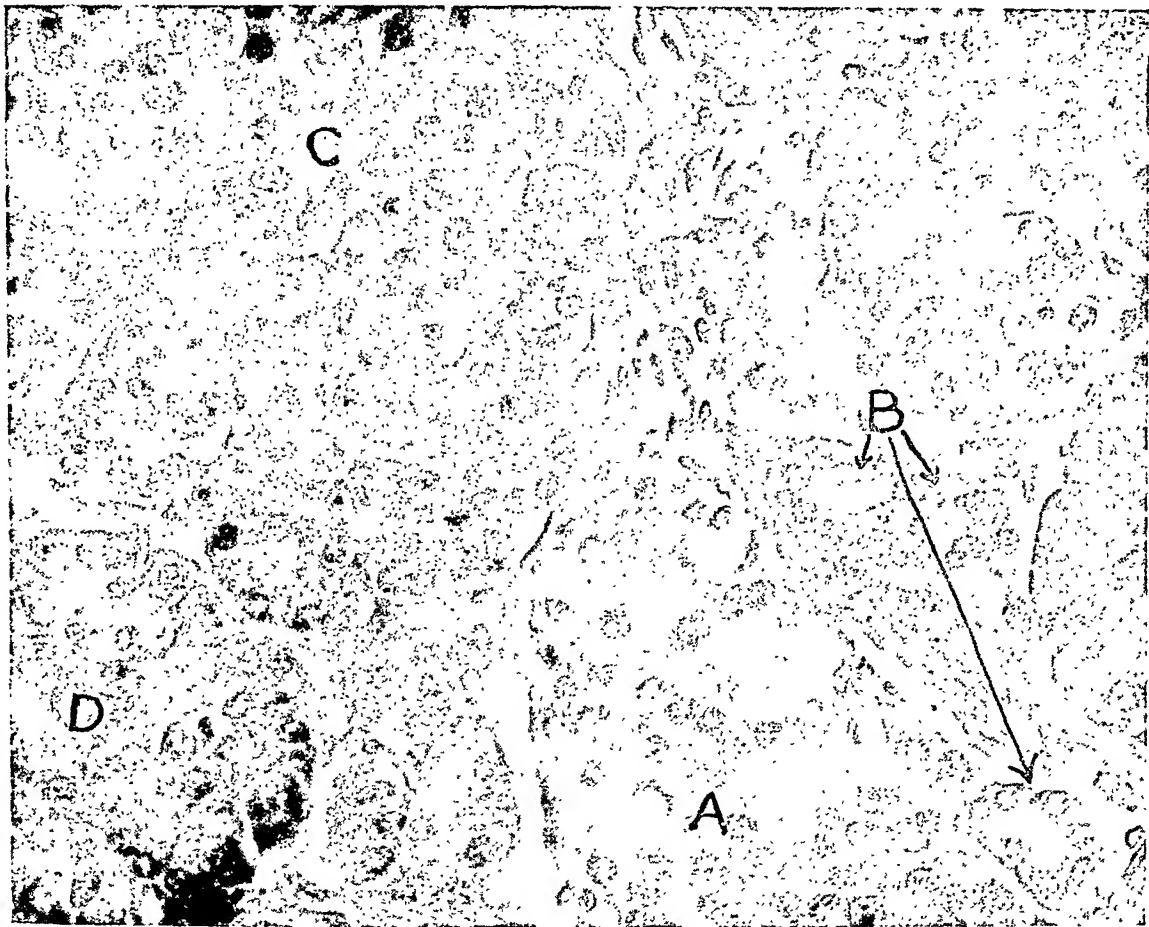


PLATE 84

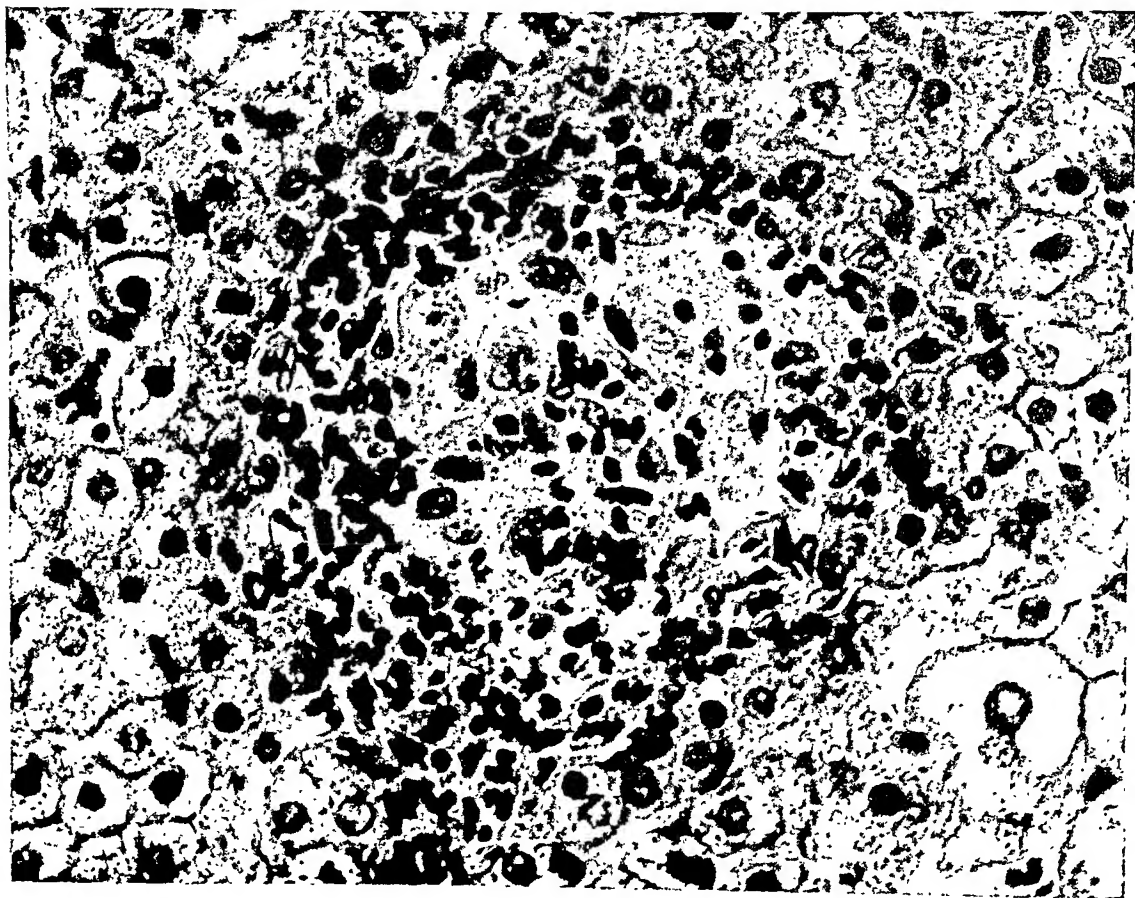
FIG. 6. Photomicrograph showing young peripheral tumor cells, (A); duct epithelium, (B); relatively undamaged islet of Langerhans, (C); and acinar epithelium, (D). The young neoplastic cells resemble duct epithelium. Faint cytoplasmic stippling of tumor cells represents beginning beta-like granulation. The tumor cells appear in close relation to duct structures. Gomori's chromium hematoxylin-phloxine stain. $\times 125$.

FIG. 7. Tubercle-like epithelioid focus in the liver apparently due to alloxan. Mallory-Heidenhain azan stain. $\times 125$.

6



7



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METASTATIC CALCIFICATION PRODUCED IN DOGS BY HYPERVITAMINOSIS D AND HALIPHAGIA *

R. M. MULLIGAN, M.D., AND F. L. STRICKER, M.D.

*(From the Department of Pathology, University of Colorado, School of Medicine,
Denver 7, Colo.)*

The observation¹ of widespread metastatic calcification in a man who had ingested much vitamin D and alkaline salts during several months led to a review² of the literature concerning metastatic calcification and to experiments on dogs in an attempt to reproduce the lesions noted in the patient.¹

MATERIALS AND METHODS

Sixteen clinically healthy and mature mongrel dogs (15 males and 1 female) were employed in experiments made during 1946 and the first quarter of 1947. These animals were on a stock diet of dog chow containing 725 units of vitamin D₃ as fish liver oil and 250 units of vitamin D₂ as irradiated yeast per 500 gm. The calcium content was 2.4 per cent; the phosphorus, 1.8 per cent; the carbohydrate, 55.3 per cent; the protein, 22.7 per cent; the fat, 4.2 per cent; fiber, 4.75 per cent; and moisture, 9 per cent; according to an analysis furnished by the manufacturer, L. B. Dean and Company of Denver. Water was freely available at all times. In our kennels dogs have been kept on this ration for as long as 3 years without evidence of dietary deficiency and in a good state of health, weight, and activity. The supplemental vitamin D was in the form of ertron, an electrically activated ergosterol. The supplemental alkaline salt mixture (*sal hepatica*³) contained the phosphate, bicarbonate, sulfate, and chloride salts of sodium. Seven dogs received both vitamin D and the alkaline salts; 5, vitamin D alone; 2, the alkaline salts alone; and 2, the stock diet only. The

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manner of death, the number of days of each experiment, the body weight of each animal at the beginning and end of the experiments, the percentage of original body weight lost, the total international units of vitamin D₂, and the total grams of sal hepatica have been summarized in Table I. Both substances, given orally, were incorporated in small boluses of ground beef. The vitamin D was usually given in doses of 100,000 units daily, except: (a) in the first week in dogs 1 and 2 when 50,000 units daily were administered, (b) when temporary meat shortages occasionally hindered dosage for 1 to 2 days or even a week, and (c) in dogs 20 and 21 which received 150,000 units of vitamin D daily. The alkaline salts were given in doses of 7.5 to 8 gm. daily. The predominant breed in each dog used was estimated as follows: dog 1, beagle hound; dog 2, cocker spaniel; dog 7, bulldog; dog 16, poodle; dog 20, Dalmatian; and the other 11, terriers of various types (8 fox, and 1 each Scotch, wire-haired, and Airedale).

The tissues studied were obtained at autopsy immediately or within a few hours after death. The thyroid, parathyroid, and thymus glands, spleen, liver, gallbladder, pancreas, left adrenal gland, urinary bladder, prostate, testes, lymph nodes, ribs, vertebrae, and pituitary gland were fixed in Zenker's fluid. The heart, aorta, lungs, esophagus, stomach, intestines, right adrenal gland, kidneys, and brain were fixed in a 4 per cent solution of formaldehyde, so that any possible interference of potassium bichromate or mercuric chloride would be avoided in determining calcification of these viscera. The tissues were washed, trimmed, dehydrated and cleared in dioxane, imbedded in paraffin, sectioned at 8 μ , and stained with hematoxylin and eosin.

RESULTS

CLINICAL FINDINGS

All dogs given vitamin D, alone or with alkaline salts, lost 32 to 61 per cent of their original body weight. Inappetence was distinctly in evidence before each experiment was half completed and hypodipsia, apathy, and terminal coma were observed in those dogs which died. Occasionally, hematemesis or melena was noted. The dogs receiving only alkaline salts, or on the stock diet only, showed no significant changes in body weight and maintained both appetite and general activity. By the mid-period of each experiment, and usually before, the dogs on vitamin D alone exhibited small, hard, dark brown stools as contrasted with the bulky, semiliquid, unformed, light tan stools of the dogs on alkaline salts alone. Those dogs given both vitamin D and alkaline salts had moderate-sized soft, formed, light brown stools.

TABLE I

Data on Development of Costochondral Junction, Mode of Death, Number of Days of Each Experiment, Weight in Kg. at Beginning and End of Experiment, Percentage of Weight Lost, the Total Amount of Vitamin D in Millions of Units, and the Total Amount of Sal Hepatica in Gm.

Dog	Costochondral junction	Mode of death	Days	Weight		Weight loss per cent	Vitamin D million units	Sal hepatica gm.
				Beginning kg.	End kg.			
4	Cartilage open	Died	36	7.0	4.5	36	3.4	272
13	Cartilage open	Sacrificed	26	9.5	5.5	42	2.0	128
15	Cartilage open	Died	37	6.6	4.0	39	2.5	200
1	Bony seal	Died	74	11.4	6.7	41	6.4	573
2*	Bony seal	Sacrificed	47	9.5	6.4	33	3.8	365
16	Bony seal	Died	83	13.2	5.0	61	7.6	570
19	Bony seal	Died	52	8.6	4.1	53	4.6	345
6	Cartilage open	Died	19	8.0	4.8	40	1.7	
21	Cartilage open	Died	30	11.0	7.5	32	4.35	
7	Bony seal	Sacrificed	52	8.0	4.3	46	5.1	
9	Bony seal	Died	54	10.2	4.5	56	5.4	
20	Bony seal	Died	54	13.0	6.4	51	8.1	
10	Bony seal	Sacrificed	65	8.4	8.6	+2		451
11	Bony seal	Sacrificed	68	9.1	9.1	0		475
8	Bony seal	Sacrificed	52	12.5	12.0	4		
12	Bony seal	Sacrificed	61	9.5	9.3	2		

* Female.

All three groups had stools unlike the moderately firm, well formed, medium brown stools of the 2 control dogs and of many others on the stock diet observed in our kennels.

AUTOPSY FINDINGS

The gross features in the dogs receiving vitamin D alone or in combination with alkaline salts included severe wastage of the adipose tissues; dryness of the serous membranes; very small thymus glands, lymph nodes, and spleens; the frequent occurrence of one to three soft calcific plaques, 1 to 8 mm. in diameter, in the walls of one or more sinuses of the aortic valves; three to six flecks of soft calcium, 0.5 to 5 mm. in diameter, in the endocardium of the posterior wall of the left atrium; scanty, tan or dark red fluid in the stomach and intestines; small parathyroid glands, prostate, and testes; a thin, pale yellow line of calcium at the corticomedullary junction of the kidneys in 4 dogs; and pale tan marrow in the ribs and vertebrae of some dogs. Calcification in the lungs and stomach was not grossly visible or palpable, although the deposits in the splenic capsule of one dog and in the pulmonic arteries of 2 dogs were easily seen. The brains of all dogs were grossly normal.

Microscopic Calcification

The most important microscopic features were the calcium deposits in the endocardium of the left atrium, the walls of the aortic valvular sinuses, the walls of the alveoli and alveolar ducts of the lungs, the loops of Henle in the kidneys, and the stroma around the glands of the mucosa of the fundus of the stomach. To a certain degree, calcium casts in the renal collecting tubules were noteworthy and a few miscellaneous calcific deposits should be mentioned. All of these features have been included in Table II.

Heart. The calcific deposits in the endocardium of the left atrium first affected the elastic fibrils and then were spread out in granular aggregations (Fig. 1) and impinged on the inner aspect of the myocardium. No calcium was found in representative sections of either ventricle, the septum, or the right atrium.

Walls of Sinuses of Cusps of Aortic Valve. The intima (Fig. 2) was predominantly affected, but the media was focally involved, both layers showing elastic fibrils incrustated by calcium salts which were dispersed between them.

Lungs. The walls of the alveoli and alveolar ducts showed clearly defined plates of calcium of varying thickness (Fig. 3). When the

TABLE II

Calcification in Endocardium of Left Atrium of Heart, in Walls of Sinuses of Cusps of Aortic Valve, in Alveoli and Alveolar Ducts of Lungs, in Henle's Loops of Kidneys, in Stroma of Mucosa of Stomach, in Lumen of Renal Collecting Tubules, and in Other Sites

Dog	Left atrial endocardium	Aortic valvular sinuses	Lungs	Henle's loops	Stomach	Renal collecting tubules	Other sites and grade
4	grade II	grade I	grade o	grade o	grade o	grade I	None
13	o	I	o	o	o	II	None
15	o	I	o	III	III	III	Bronchial cartilages, I
1	I	I	V	o	o	I	Splenic capsule, V; pulmonary veins and arteries and bronchial cartilages, III
2	II	I	IV	o	o	II	Pulmonary arteries and bronchial cartilages, II
16	o	III	o	IV	III	II	Pulmonary arteries, III
19	I	III	V	I	III	III	Pulmonary arteries, IV; pulmonary veins and bronchial cartilages, I
6	o	I	o	I	o	II	None
21	I	II	o	I	o	II	None
7	I	II	II	o	o	I	Bronchial cartilages, I
9	o	III	o	V	IV	I	None
20	o	o	o	III	III	III	None
10	o	o	o	o	o	I	None
11	o	o	o	o	o	II	None
8	o	o	o	o	o	I	None
12	o	o	o	o	o	I	None

Grade I = minimal; Grade V = maximal; Grades II to IV = intermediate; o = absent.

veins were calcified, the intima was chiefly affected, but the arteries showed calcium deposits mainly in the adventitia and the outer aspect of the media in relation to elastic fibrils. Bronchial cartilages were unevenly marked by plaques of calcium, which largely obliterated both ground substance and chondrocytes. Calcium involved the intima of the pulmonic artery in dog 16 and the adventitia and outer media in dog 19. The walls of the sinuses of the pulmonic valve also showed intimal calcium deposits in dog 19.

Kidneys. So far as could be determined, the principal deposition of calcium in the kidneys was in the basement membranes, cells, and lumina of Henle's loops (Fig. 4), apparently preponderantly in the broad or ascending limbs. Occasional calcific casts were noted within the distal convoluted and connecting tubules. In dog 20 only, calcium was observed in the basement membranes of several glomerular capsules and within the corresponding capsular spaces. The finding of calcium casts in the collecting tubules of all 16 dogs was of equivocal significance, although dogs 15, 19, and 20 exhibited also calcification of the basement membranes and cells of several collecting tubules.

Stomach. The stroma around the glands of the middle third of the mucosa of the fundus was distinctly saturated with calcium (Fig. 5) in 5 dogs. The parenchymal cells rarely were calcified.

Other Sites. The splenic capsule of dog 1 was widely inundated by calcium salts, conspicuously precipitated on elastic fibrils. The medium and small arteries in all viscera examined, except those of the lungs, lacked calcium demonstrable by hematoxylin.

Other Microscopic Observations

Testes. The tubules were reduced in size and the stroma was relatively prominent. The seminal epithelium often consisted only of a layer of spermatogonia, as seen especially in dog 1 (Fig. 6) and dog 16, which lived longest. It was intact and spermiogenesis was greatly reduced only in dog 6 which survived the shortest time. Definite evidences of atrophy of the seminal epithelium in the other dogs which received vitamin D alone or with alkaline salts included the complete absence of spermia, only rare tubules with the epithelium developed to the spermatid stage, and most tubules lined by spermatogonia or by spermatogonia and varying numbers of primary and secondary spermatocytes, usually the former. The testes of both dogs on alkaline salts and of the two control dogs (Fig. 7) were normal and active. The data concerning the testes, the prostate, the costal and vertebral marrow, the lymphoid tissue, and the fat tissue have been collected in

TABLE III
Data on Testes, Prostate, Bone Marrow, Lymphoid Tissue, and Fat Tissue

[illegible]

Table III. The genitalia of the female dog, no 2, were normal and in the stage of anestrus of what was at least its second estrual cycle.

Prostate. Of the 11 male dogs on vitamin D alone or in combination with alkaline salts, the prostate gland showed advanced atrophy in 7 and partial atrophy in one. Three had incompletely developed prostates which could not be accurately evaluated for atrophy. The atrophic changes (Fig. 8) included decreased height and size of the epithelial cells of both ducts and acini, especially of the latter, with shrinkage of the cytoplasm and condensation of the nuclei. The acini and ducts were flattened and slit-like, and sometimes the lumina were practically obliterated. The stroma, also shrunken, was relatively increased as compared to the parenchymal elements. These atrophic changes were clearly in contrast to the normal state (Fig. 9).

Ribs. In order to correlate skeletal and genital growth, special attention was paid to the development of the costochondral junctions, two of which from each dog were examined histologically after decalcification in 5 per cent nitric acid. As indicated in Table I, dogs 4, 13, 15, 6, and 21 showed an open line along the proximal edge of the cartilage (Fig. 10), whereas all other dogs, including the 2 control animals, showed a bony seal along this edge of the cartilage whether the bony cortex of the distal end of the rib was incomplete (Fig. 11) or complete (Fig. 12). The implications of this will be discussed. To supplement these observations, the prostate, testes, and costochondral junctions of 15 other dogs, normal mature males, have been studied. Twelve showed a bony seal at the proximal edge of the cartilage of the costochondral junction and had normal testes, but only 11 had normal prostate glands, the twelfth dog having a partly developed prostate. The other 3 had open cartilage at the costochondral junction and normal prostate and testes. Since genital maturity precedes skeletal maturity by at least 10 years in man, as demonstrated by cessation of growth at the costochondral junctions, the supposition that a similar relationship holds in the dog is not unreasonable, allowing for the difference in the life spans of the two species. From the evidence noted in the 15 additional control male dogs so far examined, the finding of a bony seal at the cartilage of the costochondral junctions of a dog indicates that his chances of having normal and active testes would be 100 per cent and of having a normal and fully developed prostate would be well over 90 per cent.

Parathyroid Glands. The parathyroid glands of dogs receiving vitamin D alone or in combination with alkaline salts were smaller than normal glands. The cells were packed more closely, showed even less

distinct cytoplasmic borders than the usually hazy outlines, and contained contracted nuclei with condensed chromatin and nucleoli which were often obscured (Figs. 13 and 14). The stroma and blood vessels were relatively more conspicuous. Measurements by a standardized micrometer ocular have been made on 50 representative nuclei of the parathyroid glands of each of 30 normal dogs. As shown in Table IV, the parathyroid nuclei of the dogs receiving vitamin D alone or in combination with alkaline salts were significantly smaller than those of the 30 normal dogs.

TABLE IV

Statistical Comparison between the Size of the Nuclei of Parathyroid Glands in 30 Normal Dogs and in 12 Dogs Given Large Doses of Vitamin D

	Range	Sum of values	Mean	Sum of squares of values	Sigma	Standard error of mean
Normal (30)	μ 5.8-6.5	1855	μ 6.2	114803	0.20	0.0365
Experimental (12)	4.3-5.7	640	5.3	34302	0.37	0.1068

Standard error of difference = 0.113.

Statistical significance = 7.5.

Bone Marrow. The bone marrow was studied qualitatively by Giemsa staining. For the 12 dogs on vitamin D alone or in combination with alkaline salts, the marrow cell/fat cell ratio was reduced in 8 and increased in 2. The 2 sacrificed, which showed this ratio within normal limits, might have demonstrated a reduction had they died spontaneously. The myeloid/erythroid ratio was increased in 10 of 12 dogs, decreased in one, and normal in one. In the 10 animals with an increased myeloid/erythroid ratio, the neutrophilic granulocyte line was within normal limits in respect to composition, or was moderately shifted to the left. Eight of the 12 dogs showed atrophic fat cells in the bone marrow and 4 exhibited normal fat cells. The fat cells showed shrinkage, loss of lipids, and increasing trend to a central position of their nuclei. The space which they formerly occupied was filled by basophilic fluid, fine granules, or filaments. The vertebral marrow of an experimental dog so affected (Fig. 15) contrasted strikingly with that of a control dog (Fig. 16).

Bones. The ribs and vertebrae were studied routinely after decalcification in 5 per cent nitric acid. Dogs 9 and 20 displayed well developed osteoporosis (Fig. 17) in the vertebrae as well as in the ribs. This consisted of widening of Volkmann's and haversian canals, filling of them by increased marrow, both red and yellow, a disappearance of

osteocytes from the bone at the margins of these canals, a peeling away of granular calcium and fragmented lamellae of damaged bone, a thinning of the cortex, and a widening of marrow spaces in the medulla. Osteoclasts were absent.

Qualitative Chemical Analysis of Calcium Deposits. The more abundant deposits, which were seen microscopically in sections stained with hematoxylin and eosin as dark blue granular or homogeneous masses and plates, were presumed to be calcium salts. These were analyzed by qualitative tests previously employed.¹ The walls of the aortic sinuses in dogs 16, 19, and 9, the lungs of dogs 1, 2, and 19, the kidneys of dogs 15, 16, 9, and 20, the gastric mucosa of dogs 15, 16, 19, 9, and 20, the splenic capsule of dog 1, and the pulmonic artery of dog 19 all contained calcium phosphate by these tests. Carbonate was absent from all of these deposits. Especially interesting was the negative reaction for carbonates in the lungs of dogs 1, 2, and 19, since the patient described¹ had only calcium carbonate in his lungs.

COMMENT

The choice of an activated ergosterol preparation as the source of vitamin D in these experiments was determined by the ready availability of capsules, each containing 50,000 international units of the vitamin. The employment of doses of the magnitude of 100,000 to 150,000 units daily might seem a waste of the vitamin, but determinations⁴ of the intestinal excretion by dogs after a single massive dose of an irradiated ergosterol preparation demonstrated that the vitamin persisted in the feces for several months. Also, determinations of the serum levels of vitamin D in six human subjects receiving large doses⁵ over several months indicated that several weeks were required for the highest serum level of the vitamin to be attained and suggested that the vitamin as D₂, because of the presence of an unsaturated bond,⁶ is absorbed quite efficiently under normal conditions of alimentation until and after this maximal level is reached. Other experiments^{7,8} have showed the efficacy of vitamin D of plant origin (D₂), as compared with that of animal origin (D₃), in causing calcification of soft tissues. With irradiated ergosterol and tuna liver oil, both at various dosage levels,⁷ calcification was found in the kidneys, heart, stomach, lungs, and aorta of rats, but the calcium deposits were much heavier when irradiated ergosterol was given, indicating that vitamin D₂ is more efficacious unit for unit than vitamin D₃ in causing soft tissue calcification. With both forms of vitamin D, the kidneys were the organs most involved by calcific deposits, an observation described

by several investigators.² Another study⁸ on rats demonstrated that depression of growth, mortality, hypercalcemia, and visceral calcification were much more severe in the animals getting high levels of irradiated ergosterol than in those on comparable doses of fish liver oil concentrate.

Much has been written⁹ with regard to the rôle of vitamin D in the absorption of calcium and phosphorus from the intestinal tract. Some workers have shown that the presence of vitamin D in the intestinal tract causes increased absorption of calcium and little effect on the absorption of phosphorus. Other evidence¹⁰ has indicated that the presence of the phosphate radical potentiates the absorption of vitamin D from the intestinal tract. An observation supporting the same conclusion was the increase of tissue calcification obtained by vitamin D when a high phosphorus diet was given.¹¹ These observations,^{10,11} together with the enhancement of soft tissue calcification by alkaline diets¹² and by the intravenous injection of sodium bicarbonate solution,¹³ indicate that the employment of alkaline salts in the experiments described in the present paper would be efficacious in furnishing both a high level of phosphate ions to enhance absorption of vitamin D from the intestine and also alkaline salts to augment soft tissue calcification. Several other combinations of alkaline salts which might be similarly employed are readily and commercially available. In contrast, alkaline diets alone result in relatively little soft tissue calcification.² A high calcium diet magnifies⁶ the action of vitamin D in the healing of rickets and in the production of soft tissue calcification, although this varies with the conditions of the experiment.

The intermediate products formed from the ultraviolet irradiation of ergosterol in the production of vitamin D₂, or calciferol, as well as the products formed from the overirradiation of calciferol were thought⁶ to be solely responsible for the toxic changes and tissue calcification when irradiated ergosterol was first used experimentally, while the antirachitic properties only were attributed to the calciferol. Others have demonstrated that the toxic-calcific and antirachitic activities of vitamin D₂ obtained from irradiated ergosterol are parallel, and that other products formed in the overirradiation or overheating of ergosterol were apparently harmless¹⁴ when given in the same doses and for the same length of time. The antirachitic effects of irradiated ergosterol do not come into consideration, since the dogs used in the present experiments showed neither clinical nor pathologic signs of rickets.

Several observations may be made concerning the data contained in Tables I to III. The smaller total amount of calcification in the tis-

sues of dogs 4, 13, 15, 6, and 21 with open cartilage at their costochondral junctions contrasted rather sharply with the more extensive calcification in dogs 1, 2, 16, 19, 7, 9, and 20 with bony seals at the cartilage of the costochondral junctions. This could be due to the continuing demands by the unossified cartilage of the costochondral junctions for vitamin D, so that the substance would not be entirely free, although present in excess, for the calcification of soft tissues. The presence of new-formed bony spicules in the medulla of the ribs at the costochondral junctions with open cartilage would lend support to this hypothesis, since new medullary bone was not present when a bony seal was found at the costochondral junctions.

The prime function of vitamin D is to exert its effects at areas of ossifying cartilage.⁹ The tissues of dogs on vitamin D and alkaline salts showed a greater total amount of calcification than those of the dogs on vitamin D alone. The dogs on both vitamin D and alkaline salts also had much more calcium deposited in the lungs than those on vitamin D alone. Dogs receiving both sodium bicarbonate solution intravenously and vitamin D showed increased pulmonary as well as renal calcification by quantitative analysis.¹³ The patient described previously¹ had a tremendous amount of calcium in the lungs as compared with that found in his kidneys. This finding contrasted with the large amount of calcium noted in the kidneys of 8 other patients² who received large amounts of vitamin D. The calcification of the basement membranes and cells and the calcific casts in the lumina of the proximal one-third of the ascending limbs of the loops of Henle in the kidneys in dogs 15, 16, 19, 6, 21, 9, and 20 were observed by Goormaghtigh and Handovsky¹⁵ in dogs getting large doses of vitamin D₂. They also found calcific casts in the lumina of the distal convoluted, connecting, and collecting tubules.

Harrison and Harrison¹⁶ have found, more pronounced in young dogs, a great increase in the tubular reabsorption of phosphate when vitamin D was given. With this increased absorption of phosphate and a high level of calcium ions in the urine being concentrated, the loops of Henle of the dog would thus be especially vulnerable to calcification as indicated by the anatomic evidence in the present and previous¹⁵ experiments.

Rats, which have a similar renal structure to dogs, placed on high phosphate diets by McFarlane¹⁷ and Oliver,¹⁸ developed calcification of the cells of the ascending limbs of Henle's loops and of the cells of the ends of the proximal convoluted tubules, showing the importance of increased phosphate in the diet in causing renal calcification. The presence of calcific casts in the renal collecting tubules of the dogs

showing also calcification of Henle's loops may be explained on the basis of calcified cells being sloughed in aggregates from these loops and carried by the urine distally. Even in dogs 4, 13, 1, 2, and 7, in which calcium was not observed in Henle's loops, calcification of single cells in these loops may have been too minimal to be detected microscopically, but the calcified cells conglomerated into casts were visible within the collecting tubules. Similarly, the 2 dogs on alkaline salts alone could have had these casts in the same manner through increased phosphate furnished by the sodium phosphate in the mixture. The only factor possibly responsible for these casts in the 2 control dogs was the relatively low calcium/phosphorus ratio, 1.33, in the stock diet, since a 2:1 ratio approaches the ideal more closely.⁹ Casts in the renal collecting tubules have not been observed in dogs on the stock diet and used in other experiments in this laboratory. Leptospirosis was not found in any dog in the present series.

In connection with the localization of calcium deposits in the loops of Henle in these experiments, the sections of kidneys of female dogs with bile fistulas, which had been given large doses of estrone, were reviewed. The lipid deposits in these kidneys were originally thought¹⁹ to be located in the cells of the cortical portions of the collecting tubules, but now have been more accurately localized in the cells of the distal two-thirds of the ascending limbs of the loops of Henle.

Other factors favoring metastatic calcification in the tissues, including the heart, aorta, lungs, kidneys, stomach, and the miscellaneous sites summarized in Table II, have been discussed.²

The high degree of atrophy of the testes in the dogs receiving vitamin D alone or in combination with alkaline salts might be properly credited to the severe inanition noted in the last days of the experiment and at autopsy. Certainly, the atrophy of the lymphoid (thymus, spleen, lymph nodes, intestines, penile sheath) and adipose tissues could be explained on this basis. The possibility that there is a specific effect of vitamin D₂ through the stilbenoid linkage²⁰ present in its structure, similar to that in stilbestrol,²¹ must be considered. The same reasoning might also hold for the atrophy of the prostate gland seen in dogs 4, 15, 1, 19, 7, 9, and 20, although squamous metaplasia of ducts and acini caused by natural and synthetic estrogens, notably stilbestrol, was not observed. Harris and Moore¹⁴ found that male and female rats placed together for several months on a diet containing 15 per cent cod liver oil failed to reproduce, but exact reasons for this phenomenon were not given. The patient¹ on vitamin D and alkaline salts showed aspermiogenesis.

Atrophy of the parathyroid glands in the dogs on vitamin D alone

or with alkaline salts was apparently due to the vitamin itself. With hypercalcemia and hyperphosphatemia resulting from hypervitaminosis D as previously discussed,² the demands for parathyroid hormone are diminished, since the parathyroid glands are not called upon to maintain the blood levels of calcium and phosphorus by the normal degree of activity. The situation is different from that in secondary hyperparathyroidism in which the kidneys have been severely damaged first and the parathyroid glands are then stimulated to overwork hyperplasia to aid in the excretion of excessive phosphate in the blood and by their overactivity later to raise the level of blood calcium. In hypervitaminosis D, increased levels of both blood calcium and blood phosphorus are thrust upon normal parathyroid glands, as well as upon normal kidneys, so that the glands undergo atrophy of disuse. No study of parathyroid glands of animals receiving large doses of vitamin D has been found to date, so that the observations reported will require confirmation.

The decrease of the marrow cell/fat cell ratio in the bone marrow of dogs 4, 15, 1, 16, 21, 7, 9, and 20 indicates either a direct effect of vitamin D or of the inanition resulting from its ingestion in large doses. The increased myeloid/erythroid ratio, also noted in the human patient,¹ in all animals on D alone or in combination with alkaline salts except dogs 16 and 19, might be interpreted as a specific effect of the stilbenoid linkage in vitamin D₂, since the first effect of stilbestrol on the bone marrow of dogs is to stimulate strongly neutrophilic granulocytopoiesis as has been indicated.¹⁹ Bronchopneumonia, widespread in dog 21 and early in dog 20, and acute focal cystitis in dog 6, probably contributed to this neutrophilic granulocytopoiesis, but infection was not found in the other 7 dogs concerned. The atrophic fat cells in the bone marrow of dogs 4, 15, 1, 16, 19, 7, 9, and 20 were directly related to the severity of the percentage of original body weight lost, since these animals lost 36 to 61 per cent as compared to dogs 13, 2, 6, and 21 which had normal fat cells and lost between 32 and 42 per cent. The atrophic fat cells in the bone marrow probably represented a fat depot depleted very late in severe inanition. The atrophic fat cells and the serous fluid in the spaces formerly occupied by normal fat cells constitute the condition of serous atrophy of the fatty bone marrow, a term much to be preferred to "gelatinous degeneration."

Osteoporosis was seen, conspicuously in the vertebrae, only in dogs 9 and 20, which were given vitamin D alone for shorter periods than those during which dogs 1 and 16 received both vitamin D and alkaline salts. This might indicate a sparing action of an alkaline diet in respect

to visible destructive skeletal changes. Perhaps a lower daily dose of vitamin D over a longer period of time might result in more profound skeletal changes in dogs whether this substance be given alone or with alkaline salts. In rats on vitamin D over a relatively long time bony changes were striking,²² especially in the cortical bone of the diaphysis.

SUMMARY

1. Seven dogs receiving vitamin D and a mixture of alkaline salts and 5 given vitamin D alone showed inappetence, a loss of 32 to 61 per cent of original body weight, hypodipsia, and apathy. Two dogs on alkaline salts and 2 control dogs did not exhibit these features. The dogs in each of the four groups showed characteristic stools.

2. All dogs receiving vitamin D alone or in combination with alkaline salts revealed varying degrees of calcification in the endocardium of the left atrium, the walls of the aortic sinuses, the alveoli and alveolar ducts of the lungs, the mucosa of the fundus of the stomach, the loops of Henle of the kidneys, and miscellaneous sites. Five dogs with open cartilage at the costochondral junctions showed less total soft tissue calcification than the 7 dogs with bony seals at their costochondral junctions. The soft tissues of the 7 dogs on both vitamin D and alkaline salts revealed more calcification than those of dogs on vitamin D alone. This was true especially in the lungs. The morphologic characteristics of calcium in the kidneys were correlated with the physiologic mechanisms concerned. By qualitative chemical analysis calcium phosphate was found in the more heavily calcified sites.

3. Atrophy of the testes, prostate gland, parathyroid glands, bone marrow, and of the lymphoid and adipose tissues was discussed in relation to possible direct and indirect effects of vitamin D.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 85

- FIG. 1. Dog 4. Calcium deposits in endocardium of left atrium. Single elastic fibrils involved in lower half. Abundant calcium impinged on inner aspect of myocardium in upper half. Lumen at right. $\times 50$.
- FIG. 2. Dog 15. Deposition of calcium salts, mainly in intima of wall of aortic sinus. Lumen at right. $\times 50$.
- FIG. 3. Dog 1. Lung. Plates of calcium in walls of several alveoli and in wall of alveolar ducts in upper left-hand corner. $\times 125$.
- FIG. 4. Dog 9. Calcification in Henle's loops of kidney. Inner margin of cortex at top. Base of pyramid at bottom. $\times 35$.
- FIG. 5. Dog 15. Calcium deposits in stroma of middle third of fundic portion of gastric mucosa. $\times 50$.
- FIG. 6. Dog 1. Atrophic tubules in testis. Entire tubule lined by spermatogonia in upper half. $\times 175$.
- FIG. 7. Dog 8. Normal testis. Well developed seminal epithelium and numerous spermia in a tubule, less than one-half of the length of which is depicted. $\times 175$.

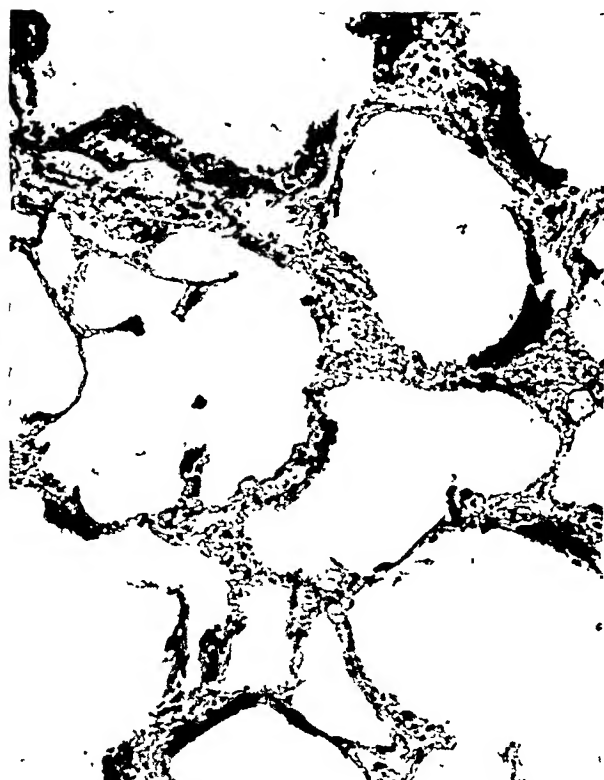
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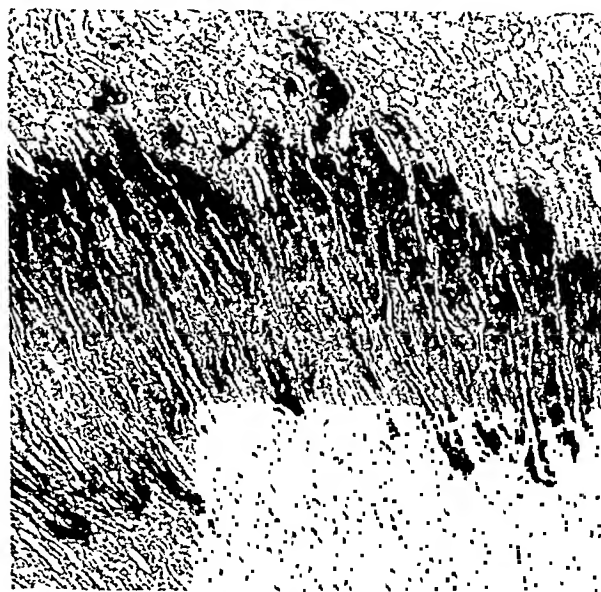
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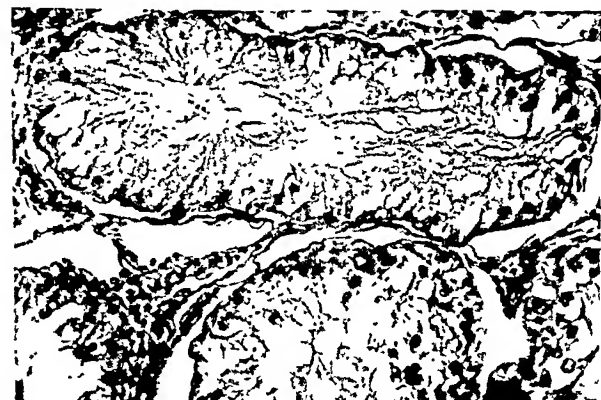
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7



Mulligan and Stricker

Calcification Produced by Hypervitaminosis D

PLATE 86

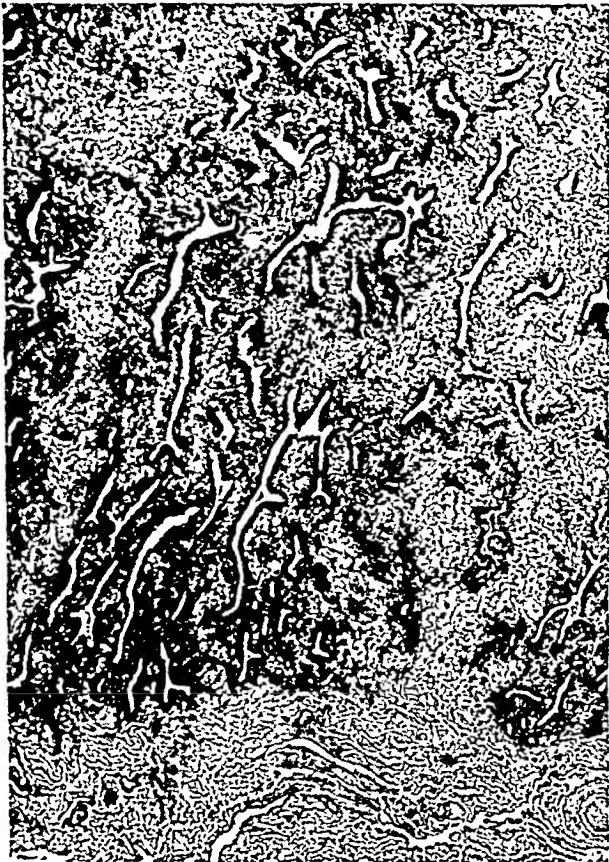
FIG. 8. Dog. 1. Atrophic prostate. $\times 50$.

FIG. 9. Dog 8. Normal prostate. $\times 50$.

FIG. 10. Dog 6. Rib, longitudinal section. Open cartilage, new-formed medullary bony trabeculae, and incomplete cortex at costochondral junction. $\times 35$.

FIG. 11. Dog 16. Rib, longitudinal section. Bony seal along cartilage and incomplete cortex at costochondral junction. Serous atrophy of fatty marrow. $\times 35$.

8



9



10



11



Mulligan and Stricker

Calcification Produced by Hypervitaminosis D

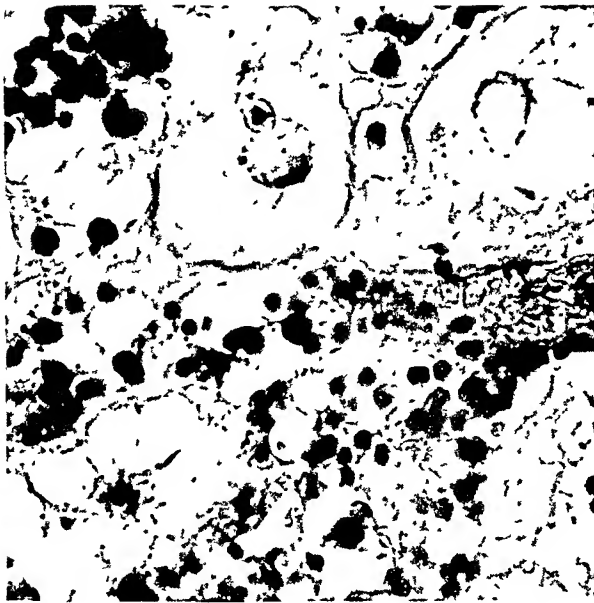
PLATE 87

- FIG. 12. Dog 11. Rib, longitudinal section. Bony seal along cartilage and complete cortex at costochondral junction. $\times 35$.
- FIG. 13. Dog 16. Atrophic cells in parathyroid gland. Nuclei shrunken and stroma and blood vessels prominent. $\times 600$.
- FIG. 14. Dog 8. Normal parathyroid gland. $\times 600$.
- FIG. 15. Dog 16. Vertebral marrow. Reduction of marrow cells. Atrophic fat cells, one well seen in lower left-hand corner. $\times 450$.
- FIG. 16. Dog 8. Normal vertebral marrow. Fat cell in lower right-hand corner. Megakaryocyte at left of center. Numerous marrow cells. $\times 450$.
- FIG. 17. Dog 9. Osteoporosis, vertebra. Widening of Volkmann's and haversian canals. Fragmented calcium in dark, irregular bands in lower edge. Widened marrow spaces and peeling away of bony lamellae. $\times 50$.

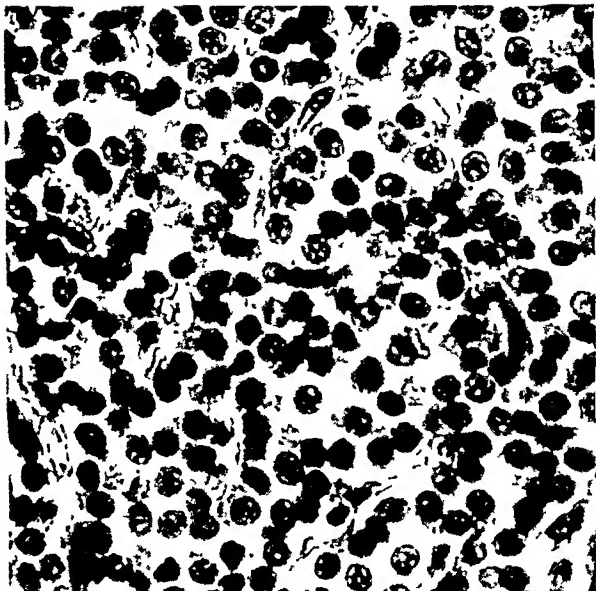
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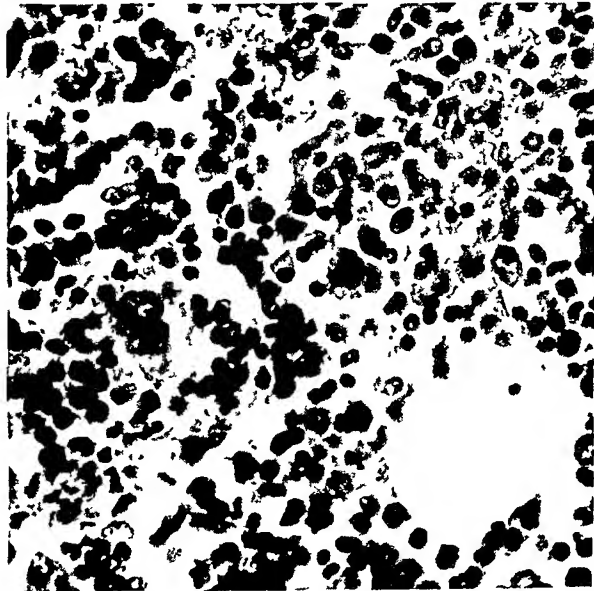
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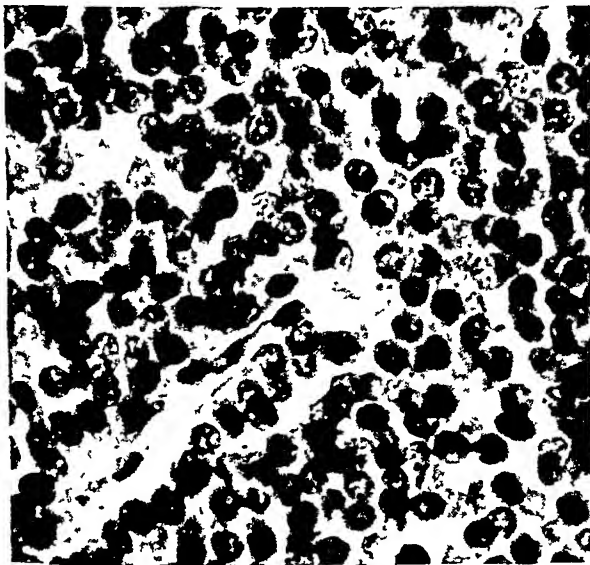
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Mulligan and Stricker

Calcification Produced by Hypervitaminosis D

CICATRIZING ENTERITIS (REGIONAL ILEITIS) AS A PATHOLOGIC ENTITY

ANALYSIS OF ONE HUNDRED AND TWENTY CASES *

SIELDS WARREN, M.D., and SHELDON C. SOMMERS, M.D.

(From the New England Deaconess Hospital, Laboratory of Pathology, Boston 15, Mass.)

Cicatrizing enteritis is a relatively uncommon¹ nonspecific inflammatory disease, usually diagnosed by exclusion of tuberculosis, syphilis, actinomycosis, and other bowel infections of specific etiology. In the voluminous literature the pathologic observations often are relegated to fine print. It is the purpose of the present article to emphasize the pathologic features, and especially the developmental stages, of cicatrizing enteritis.

HISTORICAL ASPECT

What is now called cicatrizing enteritis or regional ileitis has been described under various names for over a century. The earliest case recorded was in 1806.^{2,3} Until relatively recently there have been only sporadic case reports. Braun,⁴ in 1909, described 7 cases with inflammatory masses in the colon which had the appearance of neoplasm. These were not associated with diverticula and the cause of the inflammation could not be discovered. In 1913 Dalziel⁵ described as "chronic interstitial enteritis" 9 cases characterized by thickening of the small or large intestines. Tietze⁶ included some similar cases in an encyclopedic article on inflammatory intestinal masses. L  wen⁷ observed an unusual condition which he named "Appendicitis fibroplastica," but later⁸ recognized as a variation of cicatrizing enteritis.

Moschcowitz and Wilensky⁹ were the first to point out that many cases of nonspecific granulomas of the intestines had been diagnosed incorrectly as hyperplastic tuberculosis. The use of stricter criteria for diagnosis of tuberculosis, it was rightly predicted, would reduce the number of such cases. Mock¹⁰ provided confirmatory evidence of the nonspecificity of many of the cases called tuberculosis. He also considered that cicatrizing enteritis should be diagnosed by exclusion of inflammatory reactions of known cause. In the Continental literature,¹¹⁻¹⁵ the disease has been called "ileitis stenosans" or "ileitis ulcerosa" and emphasis placed on the diverse kinds of injury and irritation which can lead to inflammatory intestinal stenosis.

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The classic article by Crohn, Ginzburg, and Oppenheimer,¹⁶ in 1932, renamed and defined regional ileitis so well that now, 15 years later, their statements would require few alterations. Recognition of more widespread intestinal involvement,¹⁷⁻¹⁹ however, has led to proposals of inclusive terms such as cicatrizing enteritis^{20,21} and regional enteritis,²² and many others.²³⁻²⁷

TABLE I
Gross Features of Cicatrizing Enteritis in 120 Cases (Male, 60; Female, 60)

Location	Number of cases	Percentage
Ileum	112	93
Appendix		24
Proximal colon	18	15
"Skip areas"	11	9
Jejunum	5	4
Distal colon	1	
Previous appendectomy		37
Meckel's diverticulum	4	
Other diverticula	4	

Measurements of involved area	Minimum	Maximum	Average
Affected length	cm. 1.5	cm. 150.0	cm. 24.0
Thickness of intestinal wall	0.4	4.0	1.1
Diameter of lumen	0.4	4.0	1.2

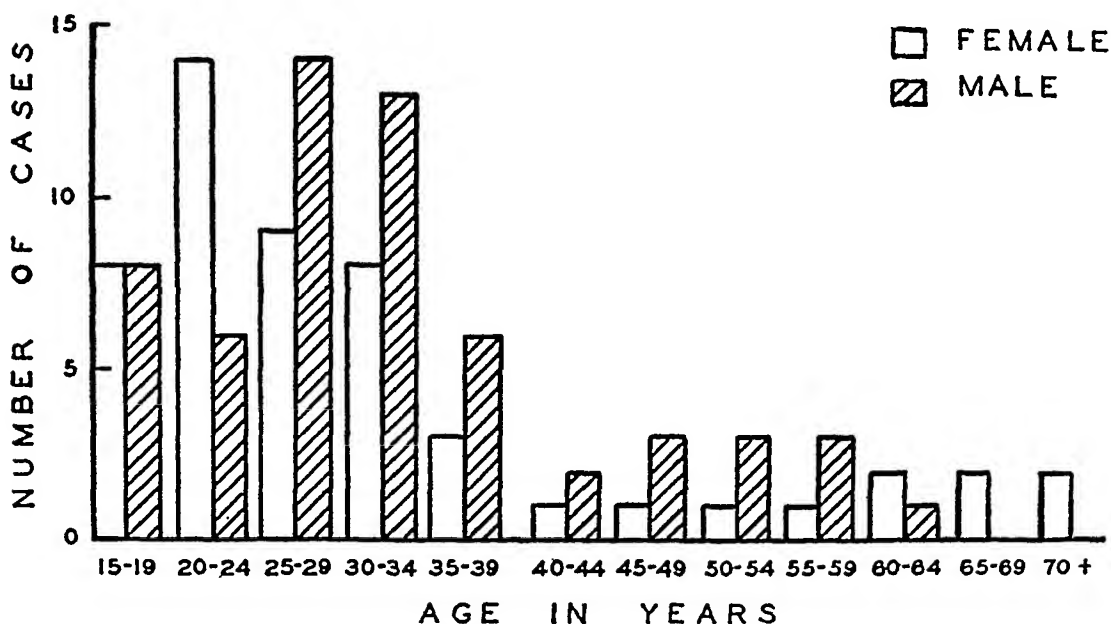
GENERAL FEATURES

Cicatrizing enteritis typically involves a single short segment of terminal ileum, and hence the term regional ileitis is justifiably popular. It is of interest that the jejunum^{28,29} and the cecum or ascending colon³⁰⁻³⁴ also are each affected in about 10 per cent of the cases. It is claimed that the disease is farthest advanced at its distal end and characteristically spreads upward,³⁵⁻³⁷ but instances of distal extension also are reported.^{31,38} The appendix may take part in the inflammatory reaction.^{30,35,39} Occasionally only the jejunum or colon is affected.⁴⁰⁻⁴⁵ The latter condition is rare, and many of the cases⁴⁶⁻⁴⁹ are open to question. Discontinuous "skip areas" of involvement in either or both the large and small intestine^{50,51} also have been described. In 4 reported instances,⁵²⁻⁵⁵ cicatrizing enteritis spread to a Meckel's diverticulum. There are no convincing reports implicating the esophagus, stomach, or duodenum.⁵⁶⁻⁵⁸ The localization and extent of cicatrizing enteritis in the present series will be found in Table I.*

*During the same period there were 240 cases of ulcerative colitis.

Most of the patients are less than 40 years old, and men and women are affected equally (Text-Fig. 1). Occasional familial cases are reported.^{59,60} There is no good evidence of special racial susceptibility. The names of patients in the present series indicate origins from all of the major stocks of the nation without any unusual predilection being apparent. One case in a Negro is included.

Cicatrizing enteritis usually is observed by the pathologist in the chronic stage, and in the present series of 120 unselected cases, 112, or 93 per cent, were of this type. Reports of more than 99 acute and 22 subacute cases were found in the literature consulted.



Text-Figure 1. Age incidence and distribution by sex of 120 cases of chronic cicatrizing enteritis.

THE ACUTE PHASE

Acute terminal ileitis is rather common, as any surgeon will testify, yet the pathologist rarely has an opportunity to examine material from this condition. Clinically, these cases are indistinguishable from acute appendicitis, but as a rule the appendix is not involved grossly. The terminal 4 to 50 cm. of ileum and its mesentery are thickened, edematous, and hyperemic. There may be a small amount of exudate on the serosa, and at times a little cloudy or clear free fluid in the peritoneal cavity. Characteristically, the segment of inflamed bowel is red-purple or maroon,^{61,62} with a sharp distal margin at the ileocecal valve and often an equally abrupt transition to normal appearing intestine at the proximal boundary. The acute form of the disease is more severe in young children, since the diameter of the bowel is smaller and edema easily produces obstruction.⁶³⁻⁶⁵

Information concerning clinical sequelae of acute ileitis is meager, but among 15 acceptable cases^{66,67} 12 patients (80 per cent) regressed, 2 (13 per cent) progressed, and one (7 per cent) died. Because of the general impression that most patients with acute ileitis recover spontaneously, the surgeon who finds this condition at operation usually either removes no tissue or performs an appendectomy.⁶⁸⁻⁷² The appendices in 30 such cases^{37,39,73-75} were described as follows: acutely or subacutely inflamed in 2 cases each, chronic periappendicitis in 10, fibrosis in 3, lymphoid hyperplasia in 15, and negative in 8 cases. Two appendiceal mucoceles were present.^{61,70} Mesenteric nodes examined in 7 cases were acutely inflamed in 4 and hyperplastic in 5 cases. A history of previous appendectomy is given in 28 per cent⁶⁸ to 50 per cent¹⁰ of all cases of cicatrizing enteritis.

Dalziel⁵ found the earliest histologic changes to be congestion, submucosal edema, and irregular hemorrhages in the intestinal mucosa and submucosa. Crohn *et al.*¹⁶ described as the primary lesions oval mucosal ulcerations about 1 cm. in diameter with axial polarity, located especially beneath the attachment of the mesentery. Ectasia of lymph and blood vessels and infiltration of polymorphonuclear leukocytes were present throughout all layers.

Erb and Farmer⁷³ reported the best documented case of acute ileitis and also the youngest: a 2½-year-old girl who died on the eighth day of illness. Salient autopsy findings included peritoneal effusion, marked edema of the terminal ileum, cecum and proximal colon, with closure of the ileocecal valve, membrane-covered ulcers on the mucosal surfaces of Peyer's patches, and enlargement of the regional lymph nodes. Microscopically, edema was most prominent in the submucosa but involved the subserosa also. The inflammatory exudate consisted mostly of large endothelial leukocytes with pale vesicular nuclei. There were also some polymorphonuclear cells and striking necrosis of lymph follicles. Some small veins were thrombosed and lymphatics were distended with granular pink material. Sinusoids of the lymph nodes were widely dilated and at times filled with large mononuclear cells.

In our series there was acute inflammation in 8 cases, but 5 of these represented acute exacerbations of cicatrizing enteritis of longer duration. The 3 regarded as true acute cases occurred in women of 22, 25, and 36 years of age. The gross and microscopic changes were similar to those described by Erb and Farmer.⁷³ The acute inflammatory exudate and edema were accompanied by numerous large, round monocyctic cells identical with Mallory's endothelial leukocytes,⁸⁰ lying free or clumped in lymphatic spaces and in interstitial tissues. The blood

vessels and their endothelial cells took no active part in these processes. Ulceration was present in 2 cases, and in one the acute reaction extended into the colon from the ileum.

THE SUBACUTE PHASE

Because cicatrizing enteritis is characterized by remissions and exacerbations usually of indefinite duration, the subacute stage is not sharply distinguishable from the acute or chronic forms.^{81,82} Histories usually describe abdominal pain and indigestion of 2 to 6 months' duration.^{83,84} Of the 6 reported cases of cicatrizing enteritis in which trauma was considered a causative factor, 4 had preoperative histories of difficulty for 2 to 4 months, and 5 showed gross and microscopic subacute cicatrizing enteritis.⁸⁵⁻⁸⁸ The ileocecal junction frequently is severely affected in the subacute and chronic stages and may be stenosed as a result of inflammation.^{16,20,25-27} The changes in the bowel, mesentery, and regional lymph nodes are more extensive and intense than those seen in the acute phase and consist of a mingling of acute and chronic characteristics. Grossly, the intestine has some of the hyperemia of the acute phase, and also the boggy generalized edematous thickening of chronic enteritis. The bowel may be very friable as a result of extensive ulceration extending through the submucosa.^{89,90} The mucosa often has a reticulated pattern produced by coalescence of ulcers, leaving scattered islands of swollen mucosa. The ulcers are covered by a fibrinous or diphtheritic membrane and the inflammatory exudate is a mixture of polymorphonuclear leukocytes, lymphocytes, and plasma cells. Eosinophilic leukocytes may be more or less numerous, but they do not characterize the subacute reaction.^{36,43,84} The proliferation of endothelial cells of the lymphatics becomes more prominent in the subacute stage and giant cell granulomas begin to appear. These will be described at length later. In the mesentery and lymph nodes there are similar but milder inflammatory reactions.

Our group includes 5 subacute cases. All occurred in women, aged 20, 20, 23, 30, and 56 years. One involved only 1.5 cm. of sigmoid colon.

THE CHRONIC PHASE

A large majority of all cases of cicatrizing enteritis have a previous history of months or years of intermittent diarrhea and cramping abdominal pain, with intestinal stenosis. Intestinal obstruction may be observed⁹¹⁻⁹⁴ but perforation with peritonitis is unusual.^{18,95,96}

The gross appearance is often characteristic enough to be diagnostic (Fig. 1). A sharply demarcated segment of intestine is indurated,

soggy, leathery, and is often compared to a rubber garden hose in size and consistency.^{6,97-101} The wall is commonly thickened to about 1 cm. with participation of all layers, and the lumen may be irregularly narrowed to 0.5 cm. or less (Fig. 2). At times the distal end of the process will barely admit a probe. Ulceration is not inevitably present, but as a rule there are shallow ulcers in the long axis of the bowel, especially beneath the mesenteric attachment. At times the ulcers are girdling or reticulated in pattern. The remaining mucosa may appear atrophic, normal, or hypertrophic and polypoid. The serosa is hyperemic, dull, and occasionally bears white pinhead tubercles.¹⁰²

Mesenteric fat surrounds the intestine to an abnormal extent. The mesentery corresponding to the segment involved is uniformly thickened, stiffened, and contains large edematous lymph nodes. The fibrosed mesenteric leaves produce irregularities in contour of the bowel, which may be slightly corrugated or distinctly shortened in "concertina" fashion.^{103,104} Intussusception is practically unknown, probably because of the restraining influence of the stiff edematous mesentery. Dense fibrous adhesions fixing the diseased bowel to adjacent structures are frequent. Proximal to the diseased portion there is usually both dilatation and hypertrophy of the bowel, occasionally with diverticular outpouchings.

Roentgenologic study of an affected ileum may show defective filling, an irregular or tapering intestinal pattern proximal to the segment most severely involved, or occasionally the characteristic "string sign" produced by a thin stream of barium passing through the stenotic lumen.¹⁰⁵

Relatively few autopsy reports of cicatrizing enteritis are extant,^{58,106-111} but data are sufficient to indicate that characteristic changes have not been found except in the involved intestine and its mesentery and regional lymph nodes. Two of our 120 cases were studied at autopsy. A boy, 19 years old, had had a 3-year history of abdominal distress, and the anatomic findings were cicatrizing enteritis of jejunum and ileum, jejunal sinus, serosal fibrous adhesions of the small intestine with angulation and partial obstruction of the jejunum, healed appendectomy, healed laparotomy incision, serous atrophy of fat, emaciation, and hyperplasia of mesenteric lymph nodes. Death was due to intestinal obstruction. The other necropsy was of a Negro woman, 37 years old, who had regional ileitis, fecal fistula, partial intestinal obstruction, ileo-ileal and ileosigmoid fistulas, a recent ileocolostomy, abdominal and inguinal subcutaneous fecal sinus tracts, ulceration and necrosis of the abdominal skin, thrombosis of the inferior vena cava and exter-

nal iliac veins, echinococcus cyst of the spleen, pigmented lesions of the skin of the face, and an exostosis of the medial epicondyle of the right knee joint. The immediate cause of death was undetermined.

The pathologic histology has usually been described as diffuse chronic inflammation and edema ending in marked fibrosis.^{10, 20, 108, 110-116} There are shallow, beveled, mucosal ulcerations coated with fibrin and inflammatory cells, including polymorphonuclear leukocytes, and beneath them the submucosa is edematous and crowded with plasma cells, lymphocytes, and eosinophils. Exudate of this type infiltrates all of the deeper layers and extends into the mesentery locally. The submucosal and subserosal lymphatics are dilated. The muscle is hypertrophied. The submucous and myenteric nerve plexuses appear prominent because the ganglion cells are swollen and the periganglionic lymph spaces are dilated.*

Focal granulomas frequently are observed scattered through the intestinal wall, in lymph nodes, or in both.^{16, 20, 100, 108, 112, 118-120} These are formed by large mononuclear phagocytes with one or more central giant cells, which are often vacuolated but usually do not contain foreign bodies. While these foci somewhat resemble those of tuberculosis, they are easily distinguishable in most cases, and acid-fast bacilli are not present. The origin and significance of the giant cells are the source of considerable disagreement. Some authors^{9, 16, 26, 113, 121-125} believe that they are foreign body giant cells developing in response to vegetable fibers, crystals and lipids of dietary origin, or parasites,¹²⁶ all of which have been carried by lymphatics from the depths of ulcers. Others^{102, 112, 113, 117, 127-130} consider these giant cells to be identical with the Langhans type.

Hadfield^{102, 127} has presented the most thorough study of the giant cells. In 13 of 20 cases of cicatrizing enteritis, he found obstructive lymphedema and lymphoid hyperplasia in the intestinal submucosa. Pale endothelial cell masses developed in the germinal centers of the lymphoid nodules and eventually replaced them completely. Giant cells then appeared in the centers of these enlarging granulomas, which he termed "giant cell systems." After reaching a certain size, the granulomas thereafter slowly regressed without showing necrosis or fibrosis. Hadfield also found "giant cell systems" in lymph nodes, even when they were not present in the intestine. This fact has not been recognized generally.^{58, 128, 131-133}

*No support has been found for a recent theory that this is the primary lesion, and cicatrizing enteritis a neuropathy.¹¹⁷ In fact, similar changes are found in ganglia of cases with intestinal neoplasms or ulcerative colitis.

In the present series there were several chronic cases which were in a stage sufficiently early to permit better evaluation of the processes involved than was possible with the few acute and subacute examples available for study. The degree of ulceration and secondary enteric infection is quite variable; often it blots out any previous pathologic change, but at times it is absent. Examination of appropriate specimens has shown the existence of sequential changes, which have not been described previously, leading to the obstruction of lymphatics and formation of granulomas in the intestinal wall and lymph nodes.

In the earliest stage, small foci of leukocytes closely resembling the endothelial cells of lymphatics appear in the lacteals of the lamina propria, between the glands and the muscularis mucosae. These endothelial cells change from flat to polygonal, with abundant granular eosinophil cytoplasm and somewhat prominent hyperchromatic nuclei. Proliferation of these cells continues and finally blocks the lymphatics (Fig. 3). In slightly later stages similar masses of proliferating endothelium obstruct lymphatics in the submucosa and subserosa (Fig. 4). Mitotic figures are found among these cells. The reaction is sharply focal and intervening stretches of the lymphatic are dilated. There is accompanying edema, but no local necrosis or inflammatory exudation; in fact the formation of granulomas is best observed in places devoid of inflammatory cellular infiltration.

Once the larger lymphatics become completely blocked deep in the submucosa and subserosa as well as in the lamina propria, eosinophils and then lymphoid cells surround these endothelial masses in increasing numbers. The endothelial cells become more closely massed and tend to coalesce, forming giant cells. Incomplete stages in this process can be found in which individual endothelial cells are partly fused. The giant cells often contain vacuoles or polymorphonuclear leukocytes, but only rare giant cells in 11 of 61 cases contained ingested foreign bodies. The vacuoles are frequently marginal and give the giant cell a scalloped appearance, making it difficult to determine whether these are fluid inclusions which do not stain for fat or cytoplasmic threads clinging to the edges of the original lymphatic and producing a false appearance of vacuolization (Fig. 5). In some cases the granulomas resemble atypical lymph follicles (Fig. 6).

Of the 120 cases, 100 (83 per cent) showed significant granulomatous response in the intestine. This was marked in 68 cases, and in 32 it required search amid the secondary inflammation to find acceptable foci. Giant cells were present in 51 specimens of intestine (43

per cent). Lymph nodes were studied in 93 cases, and 67 (72 per cent) showed endothelial response of the type described. The response was marked in 54 of these cases, and 28 (30 per cent) contained giant cells. There was no formation of granulomas at all in 12 (10 per cent) of the 120 cases, but in 4 of these no lymph nodes were isolated for study.

Necrosis within these granulomas is uncommon whatever their location, but was present in 8 of 61 cases (13 per cent). The centers are replaced by coarsely beaded red material of so-called fibrinoid appearance, and in 3 cases polymorphonuclear leukocytes also were present (Fig. 7). No caseous necrosis was observed. More commonly, these granulomas are replaced slowly by hyaline collagenous material containing scattered shrunken nuclei, and this process sometimes forms a prominent feature histologically.

An identical sequence of changes is found in the mesenteric lymph channels of the affected segment and in its regional lymph nodes (Fig. 8). The granulomas may become very prominent in these lymph nodes, and are not all of the same stage. In fact at times all variations from early thickening and partial or complete desquamation of the sinusoidal lining cells, through well formed endothelial masses, to masses partly or completely replaced by hyaline material are found in nearby nodes. Three cases of the present series had calcified mesenteric lymph nodes, and in one necrosis suggested coincidental tuberculosis. A single granuloma with giant cell was observed in the liver of one of the 2 cases autopsied, and material from a third autopsy which was studied had several similar hepatic granulomas (Fig. 9).

The outstanding secondary process which follows and often obscures the changes described is ulceration. This is not observed until edema of the intestine is well established, and at no stage do the intestinal blood vessels or the lymphoid tissue show any definite microscopic abnormalities which might predispose to the formation of ulcers. As soon as ulcers develop they are surrounded by a mixed exudate of eosinophils, lymphocytes, plasma cells, and polymorphonuclear leukocytes with a variable and usually small amount of fibrin in the ulcer bases. The ulcers extend to, or sometimes into, the muscularis, surrounded by heavy collars of granulation tissue. Nonspecific acute and chronic inflammation now merges with the distinctive primary granulomatous process and it can no longer be distinguished.

Formation of fistulas by extension of this ulceration to abdominal or perianal skin often attracts clinical attention and leads to the diagnosis

of cicatrizing enteritis.¹³¹⁻¹⁴⁰ In different series fistulas were reported in 23 to 36 per cent of the cases. In Table II is shown the incidence in our series. It is estimated that at least 10 per cent of all fecal fistulas are caused by cicatrizing enteritis.¹³⁸ Pathologic study of the tracts has revealed no special characteristics.¹³⁴ Abscesses often are formed as a by-product of the fistulas. The rarity with which peritonitis is encountered in cicatrizing enteritis is due to the slow extension of secondary inflammation, which may be retarded by the widespread lymphatic obstruction already present.

TABLE II
Complications of Cicatrizing Enteritis

	Number of cases	Percentage
Adhesions	31	26
Total fistulas:	32	27
Intestine-intestine	12	10
Intestine-skin	12	10
Into mesentery or retroperitoneum	7	6
Sigmoid-bladder	1	..

Reparative tendencies are found in chronic cases, with fibrosis of the inflamed wall, and the ulcers are covered by a thin layer of primitive or metaplastic epithelium.³⁰ The muscular layers are hypertrophied. One case of mucinous adenocarcinoma of the ascending colon, found apparently arising in intestine affected by cicatrizing enteritis, is included in the present series. The relationship may be coincidental, as there are no similar cases in the literature.

Resection is the surgical treatment of choice, and this is said to cure 83 to 85 per cent of the cases.¹⁴¹⁻¹⁴³ On the other hand, simple side-tracking operations¹⁴⁴ are successful in from 49 to 87 per cent. Attention has been drawn in the literature to postoperative recurrences of cicatrizing enteritis proximal to the resected segment in a total of 11 cases. Recurrence may become apparent clinically as late as 10 years after operation.^{145,146} One woman who died of unrelated cause 9 years after an ileocolostomy for regional ileitis had at autopsy a terminal ileum of which the lumen had been almost completely occluded by a contracted indurated mesenteric sheath.¹⁰⁹ Microscopically, this unused segment of ileum still showed chronic inflammation of the mucosal surface, edema and thickening of the wall, with muscular hypertrophy.

Medical treatment also has its adherents.^{147,148}

DIFFERENTIAL DIAGNOSIS

The problem of distinguishing between acute cicatrizing enteritis and dysentery,¹⁴⁹⁻¹⁵⁴ typhoid fever,^{155,156} amebic dysentery, "intestinal flu,"¹⁵⁷ or the effects of chemical irritants^{158,159} does not arise very often. When it does, all available clinical and laboratory data usually are required to make the proper diagnosis.

Chronic cicatrizing enteritis, in its characteristic form, is not easily confused with other lesions. Differentiation from tuberculosis is probably the most troublesome.¹⁶⁰ Intestinal tuberculosis is usually widespread, and the organisms are present in the stool. Clinically well studied cases of cicatrizing enteritis have shown no evidence of tuberculosis.¹⁰² Microscopically, the presence of granulomas with giant cells provides the main source of difficulty in distinguishing the two diseases. The granulomas of cicatrizing enteritis have a disorderly appearance with indistinct cell boundaries, the endothelial cells are mixed with lymphocytes, and the giant cells are scalloped and irregular in size and shape. Caseous necrosis is lacking and acid-fast bacilli are absent. The term cicatrizing enteritis has now absorbed almost all of the cases once called hyperplastic tuberculosis of the intestine, and the latter term is practically obsolete.^{161,162}

The resemblance between the granulomas of cicatrizing enteritis and sarcoid has been noted by many authors, some of whom have considered them morphologically indistinguishable.^{87,102,121,126,127,130-132,163} Two recent cases¹³⁰ reported as "isolated sarcoidosis of intestine" showed noncaseous tubercles in the small intestine, negative tuberculin tests, and negative stool studies for tubercle bacilli. The evidence presented, however, did not convincingly exclude them as examples of cicatrizing enteritis. Blackburn *et al.*¹⁰² failed to find positive evidence of sarcoidosis in 16 cases of regional ileitis. Analysis of 50 autopsied cases of sarcoid¹⁶⁴⁻¹⁸⁰ is of interest in this connection. Seven of these cases had some involvement of the intestine, which was tuberculous in 2,^{164,176} sarcoid generalized through stomach and bowel in 3,^{169,174,177} local sarcoid of the descending colon in one,¹⁶⁶ and an indeterminate granuloma possibly complicated by syphilis in one case.¹⁶⁸ Microscopically, the granulomas of sarcoid are larger, more numerous, and lack the lymphocytic infiltrate seen in cicatrizing enteritis. The spiculated bodies seen in the giant cells of sarcoid were absent from all of the giant cells of the 61 appropriate cases of cicatrizing enteritis in our series. Therefore at present one must reject the idea that sarcoidosis and cicatrizing enteritis are the same disease.

While the granulomas of cicatrizing enteritis are essentially different in structure from those of sarcoid and tuberculosis, they are not pathognomonic. Identical cellular aggregates sometimes are observed in diverticulitis, cancer, and lymphoma of the intestine. At least 3 otherwise typical cases of ulcerative colitis, which we have observed, contained these granulomas in the colon and regional lymph nodes.

Ulcerative colitis involves the ileum in about 25 per cent of all cases, and clinically may be difficult to distinguish from cicatrizing enteritis.^{44, 68, 181} However, the pathologist usually finds distinct differences. No matter how extensive ulcerative colitis becomes, the intestine does not undergo the marked fibrotic and edematous thickening found in cicatrizing enteritis. Microscopically, in ulcerative colitis one finds acute inflammation with fibrin, hypersecretion of mucus, edema, necrosis, sloughing of the mucosa, intense inflammation, granulation tissue, and fibrosis all taking place chiefly in the mucosa and submucosa.^{182, 183} The sluggish, progressive inflammation with tubercle-like structures of cicatrizing enteritis involves all layers of the intestine and is accompanied by more marked edema, fibrosis, and muscular hypertrophy.

Regional colitis,^{101, 184, 185} which attacks the proximal colon and spares the sigmoid, and hyperplastic colitis,¹⁸⁶ in which a localized inflammatory mass is formed, apparently do not differ pathologically from ulcerative colitis. One must, however, be prepared to recognize skip areas of cicatrizing enteritis in the proximal colon.

Amebic dysentery reaches the terminal ileum only in a late generalized stage. In the older cases fibrosis may cause considerable thickening of the bowel, but this is seldom comparable to the amount found in cicatrizing enteritis, and the distribution of the ulcerations is a useful distinguishing feature.

Bacillary dysentery affects the colon much more often than the small intestine, producing necrosis of lymphoid tissue leading to ulceration.¹⁵⁴ Chronic edematous induration of intestine and mesentery is not found as in cicatrizing enteritis. Microscopically, the inflammation in dysentery is located mainly near the mucosal surface, and there is no diffuse granulomatous process infiltrating the intestine, mesentery, and regional lymph nodes.

Lymphogranuloma venereum of the colon occasionally may suggest cicatrizing enteritis grossly.¹⁸⁷ Granulomatous inflammation is present, with tubercles the centers of which are necrotic and contain polymorphonuclear leukocytes. Elsewhere there is diffuse plasma cell

infiltration. These microscopic findings do not resemble cicatrizing enteritis. The Frei test will aid clinical differentiation.¹⁸⁸

Talc granuloma, usually caused by excess powder on gloves at laparotomy, may produce a gross appearance indistinguishable from regional ileitis, and the presence microscopically of doubly refractile crystals surrounded by epithelioid or giant cells should always suggest inquiry as to this possibility. Lichtman and co-workers^{189,190} found crystals in about 25 per cent of 198 cases at the Mayo Clinic, originally called noncaseous tuberculosis of ileum, and believed that 33 (16 per cent) contained enough doubly refractile crystals to be of etiologic significance. They listed more than thirty-five other pathogenic agents which could produce pseudotuberculous granulomas. Five of our cases contained doubly refractile material, but only one had extensive deposits. The specimen was from the jejunum of an 18-year-old girl who had no previous operation, and the origin and significance of the crystals are unknown.

ETIOLOGY

Most clinical authors have considered cicatrizing enteritis to be an infection of unknown cause,^{97,102,114,191} and bacteriologic studies of many cases have failed to reveal a specific agent. In a case of Erb and Farmer⁷³ a nonmotile *Escherichia coli* variant was isolated in pure culture from the intestine, blood, and abdominal organs. Three of their 6 patients with acute ileitis possessed serum agglutinins against this same organism in a titer higher than 1:160. Attacks of acute ileitis also have been associated with severe pharyngitis¹⁹² or diphtheria.¹⁹³ Various bacteria of doubtful pathogenicity, such as anerobic streptococci,¹⁹⁴ *Bacillus proteus*, and *Aerobacter aerogenes*^{74,195} also have been isolated. Pumphrey¹⁹⁶ commonly found no growth of bacteria from uncontaminated surgical specimens. No positive evidence of viral etiology has been reported.

Numerous other suggestions have been made. Felsen¹⁵⁰⁻¹⁵⁴ considered both cicatrizing enteritis and ulcerative colitis to be related to bacillary dysentery. Trauma and lack of good collateral circulation have been blamed.⁸⁵ Sympathetic hyperexcitability of the terminal ileum has been suggested.¹⁹⁷ Some writers have implicated allergy, partly because of the numerous eosinophils present.^{81,198} Other articles^{30,135} have stated that the disease is a complication of appendicitis, but most authors agree with Mixter¹⁹⁴ that an unoffending appendix frequently is removed. The primary site of cicatrizing enteritis is generally thought to be in the intestine,^{21,32,50,118,199} but a few writers have

concluded that disease of mesenteric lymph nodes is the cause.^{30, 101, 198, 200}

None of these ideas is entirely acceptable, and the etiology remains unknown. Pugh¹⁰⁴ remarked that absence of a known etiologic agent is a prerequisite for the diagnosis of cicatrizing enteritis. The microscopic picture is that of a response to lipid, such as is produced experimentally by injection of animal oils, according to Pinkerton.¹³¹ Fat stains of the granulomas and giant cells in 5 cases of the present series were weakly positive. The granularity of the cytoplasm of the giant cells suggested finely divided lipid. It is suggested that fat-containing chyle may escape from the damaged lymphatics and act as a chronic irritant to interstitial tissues.

PATHOGENESIS

That cicatrizing enteritis is a clinical, but not a pathologic entity^{22, 67, 111, 131, 201} has been the general opinion held by most physicians, despite claims to the contrary.^{97, 127} It is true that in the late ulcerated and fibrotic stage with its microscopic picture of banal chronic inflammation, fibrosis, abscesses, and fistulae, characteristic features rarely will be found. But observation of earlier lesions has, we believe, shown a sufficiently uniform process of special type to warrant considering cicatrizing enteritis a pathologic entity. Sections taken through the most severely ulcerated and secondarily infected portions of intestine are less characteristic microscopically than those from less damaged intestine, particularly around the proximal edge of the grossly changed segment. Likewise, the largest available lymph nodes more often show only inflammation and edema, while medium-sized nodes are richer in granulomatous endothelial and giant cell foci.

Lymphatic obstruction has been favored by many authors^{117, 143, 199, 202, 203} as the most important factor in the development of cicatrizing enteritis, but has been observed previously only by Blackburn *et al.*¹⁰² According to these authors, the primary lesion is a specific hyperplasia of lymphadenoid tissue in the submucosa and the regional lymph nodes. The lymphatics are obstructed by lymphocytes. In our series lymphatic blockade also was observed, but was the result of endothelial cell proliferation and desquamation. Superficial erosions may be induced by the marked edema and in time be followed by secondary infection, lumpy abscesses, sinus tracts, fibrosis, and adhesions. Strömbeck⁵⁰ explained the predilection of the ulcers for the mesenteric side of the bowel on the basis of fixation of the vessels by phlegmonous edema of the mesentery.

The experimental work of Reichert and Mathes²⁰⁴ adds further

evidence in support of the fundamental significance of lymphatic block. Sclerosing solutions injected into cannulated lymphatics in the intestines of dogs produced gross and microscopic changes resembling cicatrizing enteritis. Almost all of the animals showed acute lymphedema, and the lymphatics were filled and blocked by large pale cells. After 1 month, chronic lymphedema with greater submucosal thickening was observed, but the inflammatory cells disappeared. Intravenous introduction of *Escherichia coli* enhanced these changes. Sclerosis of the intramural intestinal lymphatics was not achieved; had it been, more severe mucosal changes probably would have occurred. The conclusion drawn was that both low-grade chronic infection and chronic permanent lymphedema formed the basis of regional cicatrizing enteritis.

The suggestion^{10, 104, 110, 205} that the primary lesion is a proliferation of interstitial elements which may interfere with the blood supply and thus cause necrosis, ulceration, and cicatrization does not explain the characteristic edema. Again the hypothesis of minor superficial ulcers as a portal of entry for a slowly spreading infection of low virulence^{32, 206} is open to objection, since numerous cases show marked disease of the bowel with slight or absent ulceration.

SUMMARY

One hundred and twenty unselected cases of cicatrizing enteritis have been analyzed together with a comprehensive review of the literature on the acute, subacute, and chronic stages of the disease. The terminal ileum of a young adult is most often affected, in either sex and of any race, but the jejunum, upper ileum, appendix, cecum, or colon may be involved. The etiology is unknown. The characteristic gross findings are sharply demarcated induration and edema of intestine and its mesentery, with enlargement of regional lymph nodes. Microscopic sequences indicate that swelling and proliferation of lymphatic endothelium in intestine and lymph nodes cause occlusion of lymphatics and resulting edema. Granulomas containing giant cells are formed by these cells throughout the intestinal wall and in mesentery, lymph nodes, and liver. These granulomas slowly hyalinize, usually without necrosis, or are obscured by secondary bacterial infection. In late stages, subacute and chronic inflammation, fibrosis and muscular hypertrophy of the intestine are prominent. Cicatrizing enteritis is an acceptable pathologic entity.

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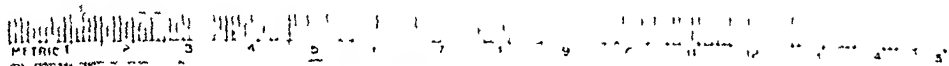
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[Illustrations follow]

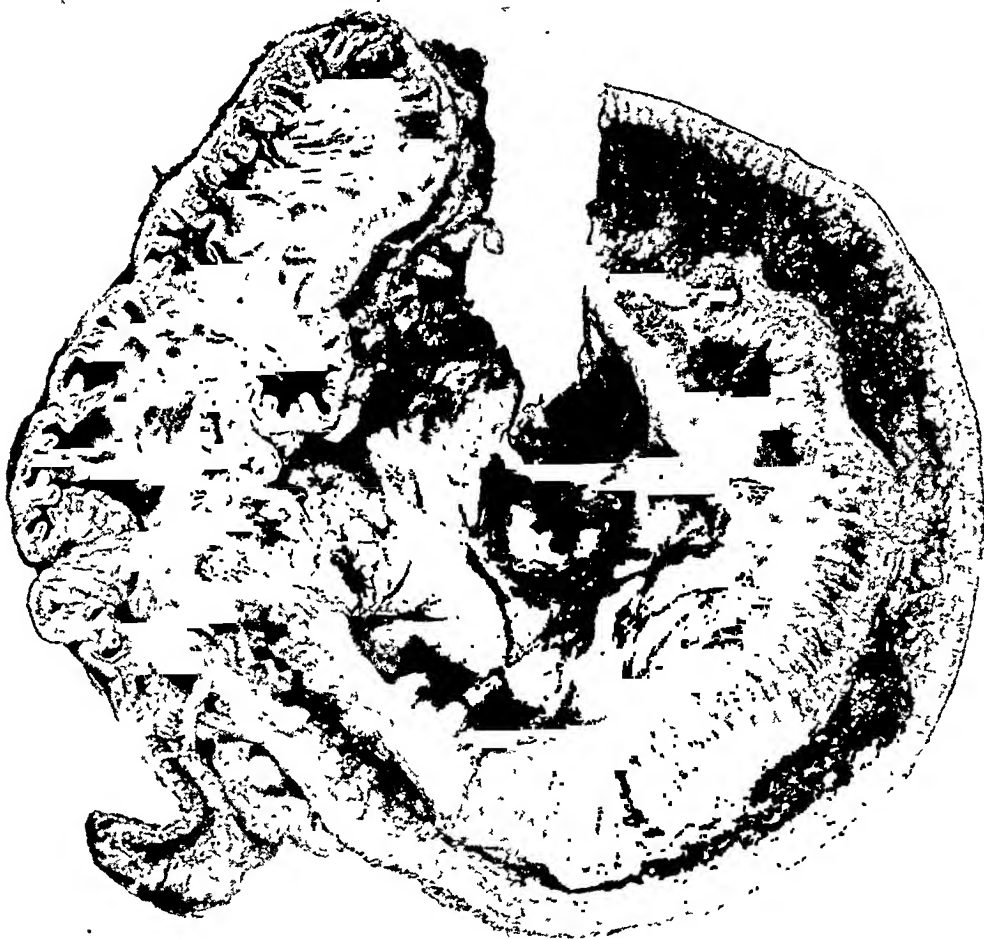
DESCRIPTION OF PLATES

PLATE 88

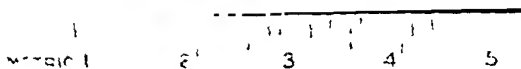
- FIG. 1. Cicatrizing enteritis of the terminal ileum, sparing the cecum and appendix. Thickening of the small intestine and its mesentery is present, with stenosis of the lumen. The patient was a boy, 16 years old.
- FIG. 2. Ileum from cicatrizing enteritis on the left. Normal ileum is on the right.
- FIG. 3. Ileum of early chronic cicatrizing enteritis, showing granulomas in the lamina propria on each side of the base of a villus. Phosphotungstic acid-hematoxylin stain. $\times 40$.



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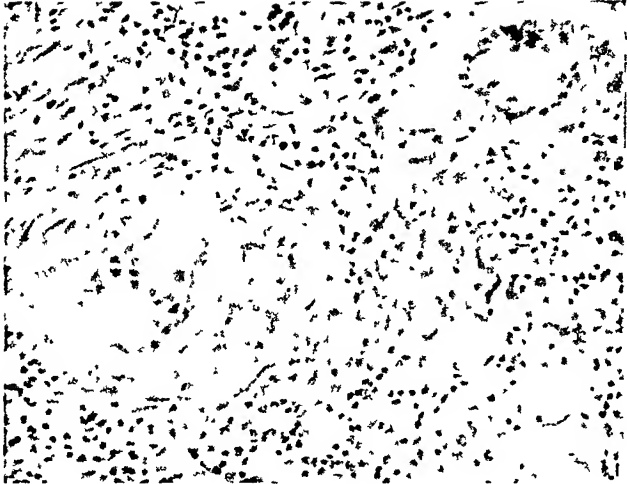
PLATE 89

- FIG. 4. Ileum in chronic cicatrizing enteritis, showing endothelial cells proliferating and blocking a submucosal lymphatic. Phosphotungstic acid-hematoxylin stain. $\times 800$.
- FIG. 5. Lymph node. Giant cells in a granuloma, showing different stages of formation, and peripheral scalloping. Phosphotungstic acid-hematoxylin stain. $\times 200$.
- FIG. 6. Fully developed granulomas in the submucosa beneath intact mucosa. These simulate lymph nodules. Phosphotungstic acid-hematoxylin stain. $\times 30$.
- FIG. 7. Lymph node. Necrosis in a granuloma of cicatrizing enteritis. The intestinal granulomas in this case also showed necrosis. Phosphotungstic acid-hematoxylin stain. $\times 125$.
- FIG. 8. Granuloma around a lymphatic in the mesentery of cicatrizing enteritis. Phosphotungstic acid-hematoxylin stain. $\times 200$.
- FIG. 9. Granuloma in the liver of an autopsied case of cicatrizing enteritis. Phosphotungstic acid-hematoxylin stain. $\times 200$.

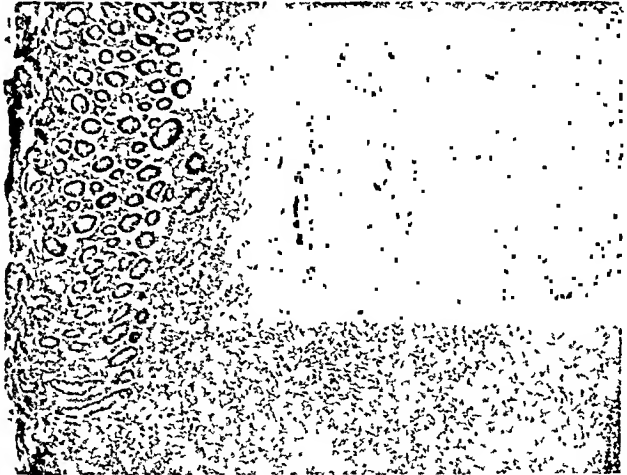
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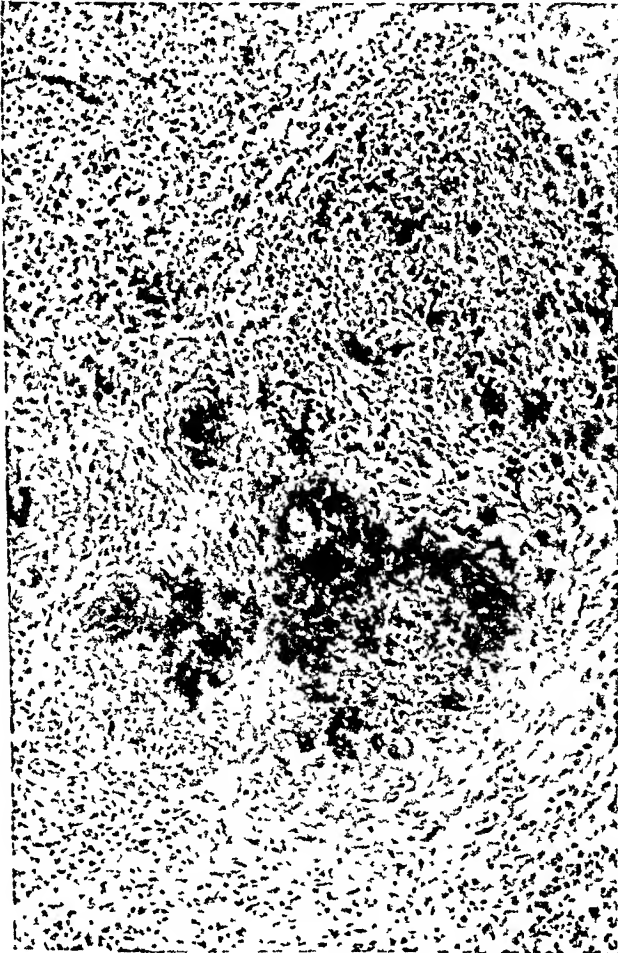
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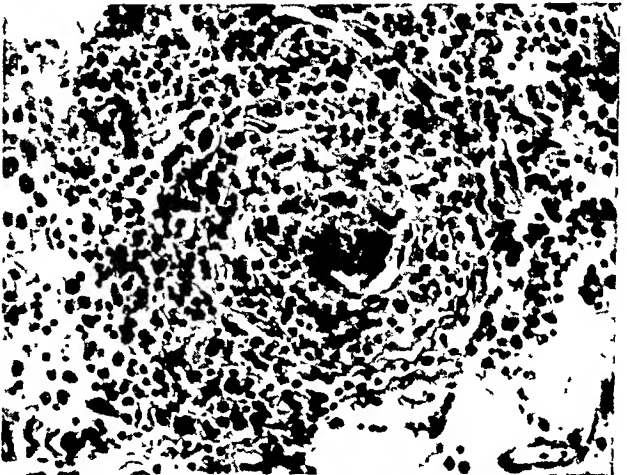
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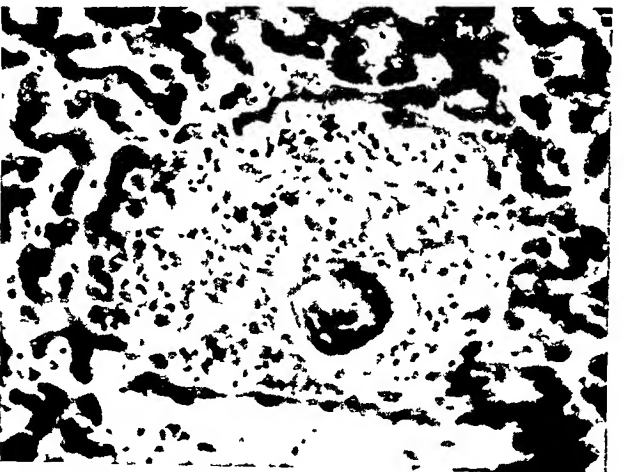
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HEPATIC AND RENAL INJURY WITH CALCIUM DEPOSITS AND CIRRHOSIS PRODUCED IN RATS BY PYRIDINE *

JAMES H. BAXTER, M.D. †

(From the Department of Internal Medicine, Southwestern Medical College, Dallas, Texas, and the Department of Biochemistry, Cornell University Medical College, New York, N.Y.)

Observations made in a series of nutritional and chemical investigations of the mechanisms of the hepatic and renal injury produced by pyridine are presented in this report. Pyridine was chosen for use in these studies because of the possibility that it might, by its methylation in the body,¹ cause hepatic and renal injury by draining the labile methyl groups from choline and methionine, and thus produce an "intrinsic" deficiency of these substances.^{2,3} The observations that pyridine produced hepatic and renal injury, which was prevented to a considerable extent by methionine,² and that the already methylated product of pyridine, in equivalent amounts, did not produce the lesions,⁴ seemed to be in favor of this hypothesis. However, because of the failure to obtain the methylated product from the urine of the animals fed pyridine, while it was readily obtained from those fed the already methylated product itself,⁴ and particularly because of the ineffectiveness of methyl-containing choline in preventing death of the animals, but effectiveness of non-methyl-containing cystine (although the efficacy of cystine was markedly enhanced by simultaneous administration of choline),³ it now appears that if this mechanism played a part in the observed toxicity of pyridine, it was not the only or most important one.⁵ Possible mechanisms of action of pyridine in causing renal and hepatic damage will be considered in other reports.

Pyridine is included in the structure of many biologically active substances, and is formed in small amounts in the burning of tobacco and the roasting of coffee. Its toxic actions, particularly on protracted administration, have not been studied extensively, and apparently there have been no investigations of the effects of its administration in the diet. Pyridine has not been regarded as a highly toxic substance by most investigators. Pollock, Finkelman, and Arieff,⁶ in 1943, reviewed the subject of pyridine toxicity and reported the effects of prolonged administration to 5 patients, 2 of whom became seriously ill,

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† Welch Fellow in Internal Medicine of the National Research Council. Now at the Hospital of the Rockefeller Institute for Medical Research, New York 21, N.Y.

apparently due to hepatic and renal damage. Pyridine usually has not been listed among the substances producing hepatic and renal injury,⁷ however, and there have been no reports of the production of cirrhosis by means of this agent.

EXPERIMENTAL MATERIALS AND METHODS

Animals. More than 300 rats have been fed diets containing pyridine, with or without other supplements, for periods up to 4 months. At the end of 4 months on the pyridine-containing diets, some of the animals were placed on stock diet and have now been observed for an additional 2 months, although few of the latter animals have been autopsied.

In most experiments, young male rats (Sprague-Dawley or Sherman) after weaning were placed for about 1 week on stock diet and then transferred to the experimental diets, some of which contained pyridine. Control groups were run in each experiment. Older rats have been used to a limited extent, with results which did not differ markedly from those obtained with young rats.

Pyridine. In the early experiments, redistilled pyridine was added to the diets and considerable care exercised to prevent loss by evaporation. In later experiments, pyridine citrate (A. D. Mackay Co.) was used, and the effects apparently were in no way different from those of equivalent amounts of pyridine itself. Pyridine also was administered by injection to small groups of animals. The effects of pyridine given daily by subcutaneous injection, and by stomach tube during a fasting period, were compared, in paired-feeding experiments, with the effects of equal amounts of pyridine given in the diet. Pyridine itself, rather than pyridine citrate, was used for the injections, because of the severe irritating effect of the latter agent.

Diets. In the beginning, because of the idea expressed above concerning the mechanism of action of pyridine, it seemed desirable to use an experimental diet low in choline and methionine, but not so low that pathologic lesions would result from the diet alone, during the experimental period. Later, this diet seemed undesirable because in spite of control groups, it was difficult to be certain that the inadequate, or at least suboptimal, diet was not partially responsible for the lesions observed. A diet which was higher in protein was then used, and finally a diet containing optimal levels of casein and choline was adopted. The biologic value of this third diet was not increased by further additions of casein and choline.

The compositions of the diets are shown in Table I.

Choline Content of Diets. Diets 1 and 2 contained no added choline. The yeast, starch, and casein per kg. of these diets contained approximately 250 mg. of choline as determined by Glick's modification⁸ of the Reineckate method. On these low-choline diets, young animals which were above the most susceptible age never died with the hemorrhagic-kidney syndrome⁹ of choline deficiency, apparently because of the small food intake and an effect of the starch which has been investigated more fully and reported elsewhere.¹⁰ Animals on diet 1, and to a

TABLE I
Composition of Experimental Diets

	No. 1	No. 2	No. 3
Casein*	10%	18%	25%
Lard	20	20	—
Hydrogenated vegetable oil	—	—	19
Corn oil	—	—	1
Sucrose	30	22	51
Corn starch ("Argo")	29	29	—
Salt mixture (Osborne and Mendel†)	4	4	4
Yeast	5	5	—
Cod liver oil	2	2	—
Vitamin supplements and choline	—	—	+

Supplements per kg. of diet 3			
Thiamine chloride	20 mg.	Para-amino-benzoic acid	10.0 mg.
Pyridoxine	20 mg.	Biotin	0.1 mg.
Riboflavin	30 mg.	Folic acid	0.2 mg.
Ca pantothenate	50 mg.	2-Methyl-1,4-naphthoquinone	1.5 mg.
Nicotinic acid	200 mg.	dl- α -Tocopherol acetate	15.0 mg.
Inositol	500 mg.	Vitamin A conc.	60,000 U. S. P. units
Ascorbic acid	500 mg.	Vitamin D conc.	6,000-10,000 U. S. P. units
Choline chloride		2.0 or 3.0 gm.	

* Only the casein used in diet 3 was vitamin free.

† Osborne, T. B., and Mendel, L. B. The relation of growth to the chemical constituents of the diet. *J. Biol. Chem.*, 1913, 15, 311-326.

lesser extent on diet 2, grew more slowly than those on diet 3, but no extensive pathologic lesions were observed during the experimental periods on these suboptimal diets (up to 2 months).

Examination of Urine and Blood. A number of animals were kept in metabolic cages and the urine collected. The specimens were examined for albumin, and in a few instances for blood, sugar, and pigments. Hemoglobin determinations were done colorimetrically on samples of tail blood from a small group of rats, before and after 2 weeks on the pyridine-containing diet. The animals were then sacrificed and the blood examined spectroscopically. Urea and sugar determinations were made on the blood of a few animals in the acute stages of illness due to pyridine.

Examination of Animals and Tissues. Animals were autopsied immediately after death or when death appeared imminent, and the organs examined grossly. Other animals were sacrificed at various intervals in order to study the different stages of injury.

Organs were then fixed in 10 per cent neutral formalin or absolute alcohol. Sections of livers and kidneys of most of the animals and sections of other organs of a few animals were examined microscopically after staining with hematoxylin and eosin. The following stains or methods also were employed in some cases: potassium ferrocyanide-hydrochloric acid for iron, Giemsa's stain, sudan IV, carbol fuchsin, Mallory's phosphotungstic acid hematoxylin, Masson's connective tissue stain, and von Kossa's silver nitrate method for calcium, with and without previous treatment of sections with 5 to 10 per cent concentrated hydrochloric acid. A few unstained sections were examined for pigments and highly refractile substances with visible light, and for fluorescent material with ultraviolet light.

Influence of Diet on Results

The results of feeding pyridine in the different diets were essentially the same, with a few exceptions. Raising the casein level increased somewhat the resistance of the animals to the effects of pyridine, so that it was necessary to increase the pyridine levels simultaneously, in order to obtain approximately the same results. The pyridine levels that have been used with the various diets in most cases were as follows: diet 1, 0.34 per cent pyridine citrate (or 0.1 per cent pyridine); diet 2, 0.7 per cent pyridine citrate; and diet 3, 0.7 to 1.0 per cent pyridine citrate.

The animals placed on diet 1 containing pyridine usually showed a decreased food intake and immediate cessation of growth, whereas those fed diets of higher protein level plus pyridine continued to eat and grow fairly normally even to the time of death. The third difference observed was the presence of more fat and the earlier development of extensive fibrosis in the livers of the pyridine-treated animals on diet 1, and perhaps on 2, than on 3. Thus it was apparently possible, by raising the choline and casein levels, to reduce the fatty changes and fibrosis, without reducing the necrosis.

Rather large vitamin supplements were used with diet 3 to insure adequate intake of vitamins even in cases in which the food intake decreased to low levels. A small number of animals were run on the same diet with yeast and corn starch added, with no appreciable difference in results. Since diet 3 was used in most of the recent studies, and

since the lesions observed on this diet undoubtedly were due entirely to the effects of pyridine, description will be limited to the results on this diet, except when otherwise specified, but it is applicable, for the most part, to the results on the other diets as well.

RESULTS

The animals ate the pyridine-containing diet fairly well and continued to grow, in many cases, at an almost normal rate. In some groups, from 50 to 100 per cent of the animals died in the first week, and in the majority of the groups, most of the animals died within 2 or 3 weeks. Occasionally a considerable number survived for longer periods. Impending death could not always be predicted by the appearance of the animal, but a feeling of coldness as from "shock," which perhaps was in part due to the loss of blood into extensive necrotic areas in the liver, signaled an early end. Survival of the early stages of pyridine treatment apparently resulted in the development of some degree of tolerance.

The principal lesions were found in the livers and the kidneys, and it is presumed that death was due to disturbances of the functions of these organs.

Urine. The urine of the animals frequently became highly colored and contained bile pigment, and sometimes appeared red. Albumin usually was present, sometimes in large amounts, at least during the periods of obvious illness. A few examinations for blood and for sugar were all negative. The urine output sometimes increased markedly in animals which became ill. Anuria, except shortly before death, was not observed.

Blood. Hemoglobin determinations done after 2 weeks on the pyridine-containing diet showed little change from the control values. Characteristic absorption bands of methemoglobin were not noted spectroscopically. Blood chemical determinations revealed normal blood sugars and elevated urea levels.

Serous Cavities. Collections of fluid in the serous cavities were fairly common. Small hemorrhages were noted occasionally beneath the serous membranes. Bile-staining of tissues was seen frequently.

Lungs. An occasional animal exhibited atelectasis of one or more pulmonary lobes. Peribronchial inflammation was noted in some sections.

Lymph Nodes. Mesenteric and peribronchial lymph nodes sometimes appeared enlarged, particularly in animals receiving prolonged treatment.

Spleen. The size of the spleen was quite variable, but in general it seemed to be enlarged in the acute stages of injury, and smaller than normal in the chronic stages. Little hemosiderin was present.

Hepatic and Renal Lesions

Early Hepatic Lesions. The livers of animals dying of acute injury after ingestion of pyridine-containing diets usually were enlarged and darker than is normal, due at least in part to an increased content of blood.

Microscopic sections (Fig. 1) revealed very extensive necrosis with partial dissolution of cells about the central veins and filling of the spaces by red blood cells, most of which seemed to be still within vascular channels. Frequently the only parenchymal cells to escape were those at the periphery of the lobules, particularly in the region of the portal triads, and these sometimes showed degenerative changes.

Animals which survived the initial stage of necrosis usually showed evidence of extensive regenerative activity along with continued injury. Some of the liver cells at the edges of the necrotic areas contained small particles in the cytoplasm which stained dark brown with hematoxylin (Fig. 2). These collections of cytoplasmic bodies were more prominent in the later lesions and will be described in connection with them. Phagocytic cells, particularly in and about the old necrotic areas, contained large amounts of yellow pigment, most of which did not give the Prussian-blue reaction and became green on treatment with acid. Some of the large phagocytes along the central portions of the fibrous trabeculae also contained hemosiderin.

Early Renal Lesions. The kidneys usually appeared swollen, grossly. Sections showed degenerative changes in the epithelial cells, most extensive in the proximal convoluted tubules, but also involving the other segments of the tubules. The cells exhibited granular swelling, hydropic degeneration, some vacuolization, pyknosis of nuclei, and sometimes obvious necrosis. Many pink-staining casts were encountered in the tubules (Fig. 3) and the same material sometimes was present in the glomerular capsules.

Later Hepatic Lesions. More chronic lesions were observed in the animals which survived for longer periods. On gross examination, the livers exhibited a fine nodularity and the cut surfaces showed a distinctly mottled appearance. Greater irregularities of the surfaces, with shrunken and raised areas, sometimes appeared, and in the final periods of study livers were seen with nodules which were considerably larger and lighter in color than those usually seen in cirrhotic livers. The

cut surfaces of these nodules appeared compact, homogeneous, somewhat translucent, and distinctly different from the remaining liver tissue.

Microscopic sections showed that the numerous small, regular nodules which were observed grossly were composed of groups of large, hyperchromatic liver cells derived by regeneration from cells about the portal triads which had escaped destruction during the acute stages of injury. The portal triads, instead of the central veins, now occupied the centers of most of the new pseudo-lobules (Figs. 2, 7, and 12); and the central veins with collapsed stroma and necrotic débris from the central portions of the original lobules, and the newly formed fibrous tissue were now compressed between the peripheral margins of the newly formed nodules of liver cells. This series of events was repeated in whole or in part, although massive necrosis of cells usually occurred only at the beginning of pyridine treatment. Finally it became difficult to relate the architectural pattern to that originally present. In addition to the increase in fibrous tissue, infiltration by lymphocytes and polymorphonuclear leukocytes was observed in some areas.

Some animals on diet 1 containing pyridine developed well advanced cirrhosis in 1 month, and the liver cells of these animals showed extensive fatty changes (Fig. 4). Most of the animals that received diet 3 with pyridine for 2 months or more exhibited unquestionable cirrhotic changes, with disorganization of the normal lobular architecture, not accompanied by much fat (Fig. 7). In most of the latter animals, the fibrosis, while generalized, was not very abundant.

Calcium-Containing Bodies in Necrotic Liver Cells. In the chronically injured livers, collections of hepatic cells containing closely packed granules or globules in the cytoplasm, staining dark brown with alum hematoxylin, were much more striking than in the earlier stages of injury, although they were not observed in every case. These cytoplasmic bodies were not highly refractile and not readily visible in unstained sections. They were smoothly oval, variable in size, and could be distinctly seen individually only with high magnification (Fig. 15). Necrotic liver cells, often still in cords, frequently remained as somewhat hyaline, eosinophilic masses without nuclei or with only nuclear fragments (Figs. 11 and 12), and the cells containing the dark particulate bodies were confined for the most part to the peripheral margins of these old necrotic areas (Figs. 12 to 15). They closely surrounded the nodules of viable hepatic cells, and, when examined under low magnification, appeared to form crescents or rings about the nodules (Figs. 12 and 13). The cells containing the bodies stained very

prominently with silver (Fig. 13) when sections were placed in silver nitrate solution and exposed to ultraviolet light, according to von Kossa's method for staining calcium deposits.* After treatment of the sections with hydrochloric acid, the bodies were no longer demonstrable. The bodies were not stained by nuclear stains of the methylene blue type. Neither the bodies nor other substances in the sections were acid-fast, or stained by fat stains after use of fat solvents, or fluorescent with ultraviolet light. The individual globular bodies often appeared darker at the periphery than at the centers.

Large Regenerative Nodules. Examination of the sections of the tumor-like nodules which developed in the later periods of the study revealed masses of large, compact, deeply staining cells with large, hyperchromatic nuclei (Fig. 16). The nodules were sharply circumscribed and no evidence of invasion or metastasis has been noted in the animals thus far autopsied. The cells composing the nodules, while different in appearance from the remaining liver cells, were evidently derived from parenchymal cells. Slight bile duct hyperplasia was noted occasionally, but nothing resembling a malignant neoplasm of this origin was seen.

Later Renal Lesions. Grossly, the kidneys from animals receiving prolonged treatment exhibited irregular surfaces, with shallow depressions and raised areas. Sections showed that the degenerative lesions were usually less marked than in the early stages, but there were additional changes of a more chronic nature. Destruction of parenchymal cells followed by regeneration occurred, often with some distortion and disruption of normal architecture, and a moderate number of calcified tubules were seen. Frequently the original epithelial lining of the tubules appeared to have sloughed into the lumina and to have been replaced by new epithelium. The tubules showed much more than normal variation in size, and many exhibited closely placed, hyperchromatic nuclei, with considerable variation in size, shape, and relative position in the cells (Figs. 8 and 9). Mitotic figures were noted frequently (Fig. 10).

Along with this destruction and regeneration of tubular cells, there appeared many dilated tubules with flat, atrophic-appearing epithelium (Fig. 8), which in extreme cases had the appearance of endothelium. The dilatation perhaps occurred earliest in the distal segments of the nephrons and in the collecting tubules but also involved the proximal

* It should be recorded that many of the specimens were fixed in formalin which had been neutralized by calcium carbonate. However, livers fixed in redistilled alcohol also showed the calcium bodies.

convoluted tubules and loops of Henle. The relationship of this process to the other changes in the kidney was not entirely clear.

Proliferation of connective tissue in limited areas was sometimes seen, but a generalized increase in fibrous tissue of a significant degree, such as occurred in the livers, was not observed. Occasional areas of granulomatous interstitial inflammation, some containing many eosinophils, were noted. In the regions of some of the angular calcium plaques of the type frequently seen in areas of old renal necrosis, there were necrotic cells containing brown-staining globular bodies similar to those seen in the livers.

Influence of Mode of Administration on the Effects of Pyridine

Because of the observation that some animals survived amounts of pyridine in single injections greater than the total amounts ingested by others which died after several days on the pyridine-containing diets, pyridine was administered by injection to a limited number of animals. The majority of a group of rats survived throughout the experimental period of 3 weeks when given twice daily, in subcutaneous injections, more pyridine than was usually consumed in the same periods by animals receiving pyridine in the diet.

The effects of pyridine given in equal amounts during each 24-hour period, by feeding in the diet, by daily subcutaneous injection, and by daily injection by stomach tube during a fasting period were then compared in three groups of rats using the paired-feeding technic, and the rats receiving the pyridine-containing diets died before the corresponding animals of the other groups in almost every case, and showed more extensive hepatic and renal injury.

DISCUSSION

Damage produced by the diets containing pyridine was apparently limited, for the greater part, to the liver and kidney, with injury of lesser degree to the spleen and perhaps to the lung and certain other organs. Except for narcosis with large doses, the effects upon the nervous system, which have been described for other species, were not observed.

The extent of the acute hepatic necrosis, even in many animals which survived, was remarkable. Whether this necrosis was produced by a direct action of the toxic agent on the cells, or was secondary to interference with the blood supply to large portions of the central parts of the lobules caused perhaps by cellular swelling, such as has been described by others under different circumstances,¹¹ was not determined. The survival of the cells about the portal triads with

regeneration from these areas, together with the observations that in most cases the necrosis was either extensive or practically absent, and that animals which survived extensive necrosis often seemed to be resistant, at least for a time, to the further development of necrosis, suggested the latter possibility.

Rather marked reduction in fatty changes and fibrosis, with no significant reduction in necrosis, caused by increasing the choline and casein content of the diet at the same time that the pyridine level was being increased, suggested, perhaps, that the necrosis and the fatty changes and fibrosis were not results of the same single disturbance. The experiments did not distinguish clearly between the effects of choline and casein, but it seemed likely that the increase in the choline level was principally responsible for the decrease in fat and fibrous tissue, while the increase in the resistance to necrosis probably was due to the change in casein content. It is possible that dietary inadequacies of these substances were contributory causes of the lesions observed with pyridine on the first two diets, although no extensive pathologic changes occurred in the control animals on these diets.

The reversal of the normal relationship between the lobules of liver cells and the blood vessels and bile ducts, with fibrosis across the central portions of the original lobules, which occurred following the acute stages of injury, was striking. It recently has been shown by injections of India ink into the portal and hepatic veins that the fibrous trabeculae in dietary and carbon tetrachloride cirrhosis in the rat are around hepatic veins rather than periportal.¹² The question arises whether the lesions observed in the present study should not be considered examples of post-necrotic scarring rather than diffuse hepatic fibrosis or cirrhosis. The difference in the development and significance of these lesions has recently been discussed by Himsworth and Glynn.¹³ Scarring in old areas of necrosis was quite prominent, particularly in the animals on diet 3, but whether all of the fibrosis occurred as a result of necrosis was not determined. The livers in the late stages of injury presented, in addition to some areas of obvious post-necrotic scarring, finely granular surfaces (Fig. 6) and fairly uniform involvement of all lobules by the fibrotic process. Whatever was the mechanism of the fibrosis, the lesions had all of the features which are usually considered characteristic of cirrhosis. Observations on the pyridine-treated animals will be continued to determine to what extent the lesions are repaired, and to see if the large hepatic nodules progress to malignant tumors.

The sequence of pathologic events observed in the kidneys of the

animals receiving pyridine was similar in many respects to that in the livers, in so far as the epithelial cells were concerned. The responses of the mesenchymal elements differed, however, in that there was no significant generalized increase in fibrous tissue.

The causes of the dilated tubules in the chronically injured kidneys were not wholly apparent. Some tubules probably were obstructed by fibrosis or calcification resulting from injury, and it is possible that others were obstructed by albuminous material and cellular débris. Localized and generalized cellular swelling, resulting in encroachment on the lumina of blood vessels and tubules, might conceivably have contributed to the production of both necrosis and obstruction.

The renal lesions were somewhat similar to, but much greater in degree, than those observed by Baxter and Ashworth¹⁴ in human cirrhosis. It was not determined with certainty whether the renal injury was produced independently or occurred as a result of the hepatic injury. As in the case of the human lesions referred to above, it is possible that shock and vasoconstrictive renal ischemia played a part in the production of renal damage.

Apparently there have been no descriptions of calcification of the type observed in the present investigation, in reports of somewhat similar hepatic injury produced by other means. Deposition of calcium in the liver, except in certain cysts, abscesses and tumors, is rare. Even in generalized calcinosis, the liver is infrequently involved. Whether there was something specific about the pyridine injury that was responsible for the calcium deposition is not known. Since the animals also had extensive renal injury with nitrogen retention, calcification may have been favored by high levels of blood phosphate. Generous amounts of vitamin D were used in the diet, but not enough to produce calcification in normal animals. Determinations of blood calcium and phosphorus, and of the phosphatase content of the injured cells might have shed light on the mechanisms of the calcification. The calcium deposition differed from that usually seen in necrotic tissue in that it was confined to individual cells, and the calcium was deposited in the form of globules, or, perhaps, about the surfaces of preformed cytoplasmic bodies. The bodies resembled in size and shape those described by Opie¹⁵ in livers injured by butter yellow, formed by the deposition of ribonucleic acid about the mitochondria.

The principle involved in the greater toxicity of pyridine when continuously present in the diet than when given in infrequent injections may be of wider and possible practical significance in the causation of hepatic injury other than that produced by pyridine. It seems pos-

sible that the difference in effects observed with the different methods of administration was due to the more continuous presence of pyridine in the tissues, when ingested at frequent intervals in the food, or, less likely, to the formation by intestinal bacteria of a substance more toxic than pyridine itself. Other substances of low toxicity, which do not produce hepatic injury by the usual methods of administration, might conceivably behave in a manner similar to pyridine if taken in the diet or if continuously formed in the body or in the intestinal tract, and result in hepatic or renal damage even when present in low concentrations.

SUMMARY

Pyridine or pyridine citrate, when incorporated in diets which did not themselves produce pathologic changes during the experimental period, caused acute hepatic and renal injury, followed by regenerative changes, cirrhosis, and chronic renal injury.

Increasing the choline (and casein) content of the diet at the same time that the pyridine level was also being increased, caused a marked reduction in fatty changes and fibrosis without any significant reduction in the severity and extent of the acute necrosis.

Large tumor-like nodules were observed in some of the livers, but no evidence of invasion or metastases was seen.

In parenchymal cells at the edges of old necrotic areas in the livers, there were accumulations of oval cytoplasmic bodies which stained dark brown with hematoxylin and gave the histochemical reactions of calcium.

Amounts of pyridine which produced death due to hepatic and renal injury when fed in the diet apparently were much less toxic when administered by infrequent subcutaneous injection or by stomach-tube.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 90

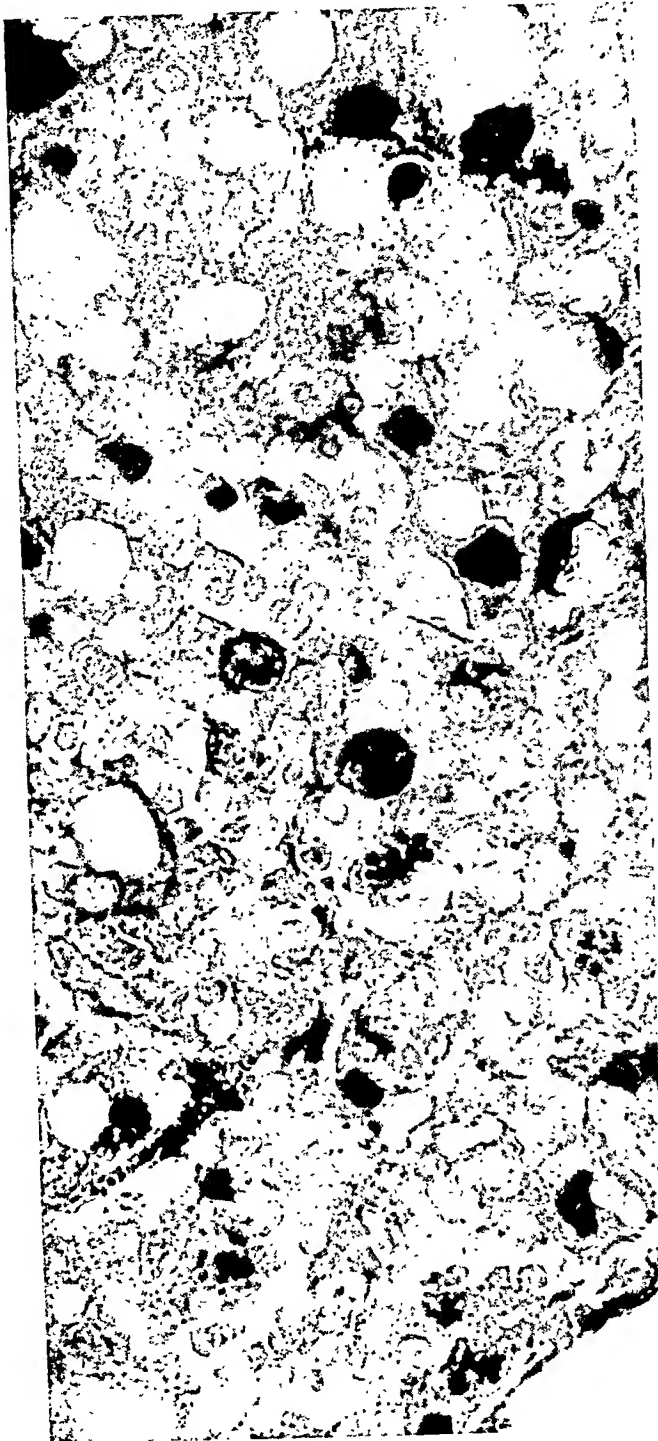
FIG. 1. Necrosis of cells about the central vein in the liver from a rat which died after 16 days on diet 1 plus 0.2 per cent pyridine. Dissolution of cells with nuclear fragmentation may be noted. Many necrotic cells remain as shells, some of which are full of red blood cells. Surviving cells in the peripheral portions of the lobule contained fat globules. Hematoxylin and eosin stain. $\times 580$.

FIG. 2. Regeneration of a liver nodule from the cells about a portal triad, in an animal sacrificed after 35 days on diet 2 plus 0.2 per cent pyridine. The portal triad is at the center of the new pseudo-lobule, with the necrotic area at the periphery. Dark-staining cells are seen at the edge of the necrotic zone. This animal almost died in the early stages of the experiment, but after the initial illness was able to continue on the same diet without serious results. Hematoxylin and eosin stain. $\times 110$.

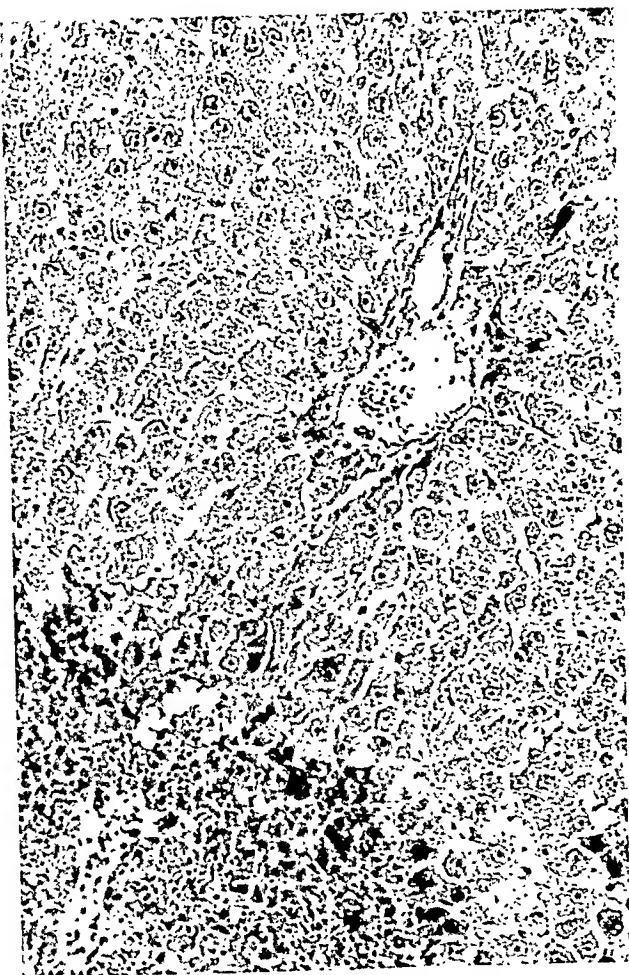
FIG. 3. Degenerative changes in the epithelium and tubular casts in the kidney from a rat dying after 3 days on diet 1 plus 0.1 per cent pyridine. Hematoxylin and eosin stain. $\times 110$.

FIG. 4. Cirrhosis of the liver with extensive fatty infiltration. The rat was sacrificed after 35 days on diet 1 with 0.1 per cent pyridine. For comparison with Figure 7. A photograph of the liver from which this section was taken is shown in Figure 5. Hematoxylin and eosin stain. $\times 110$.

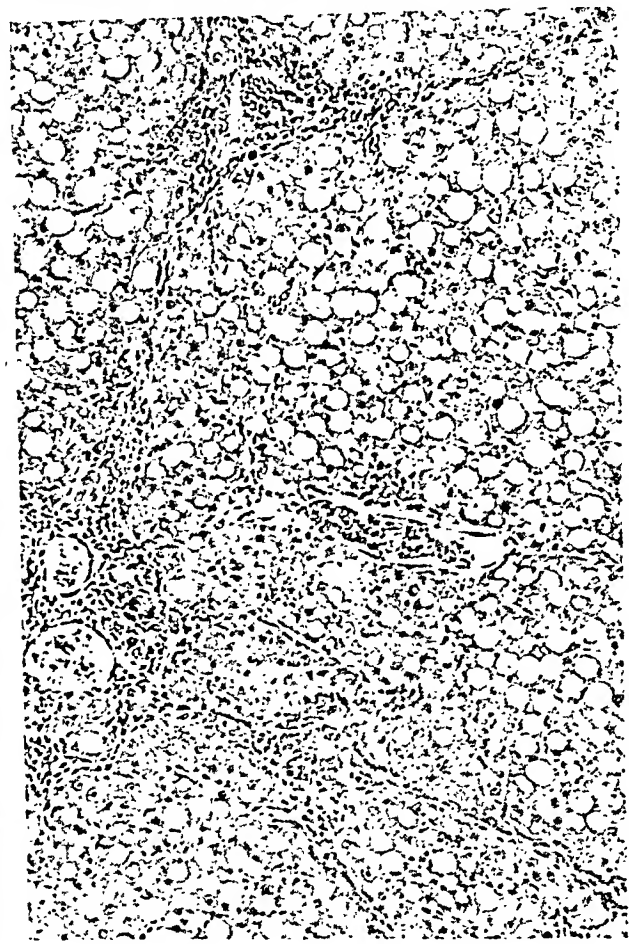
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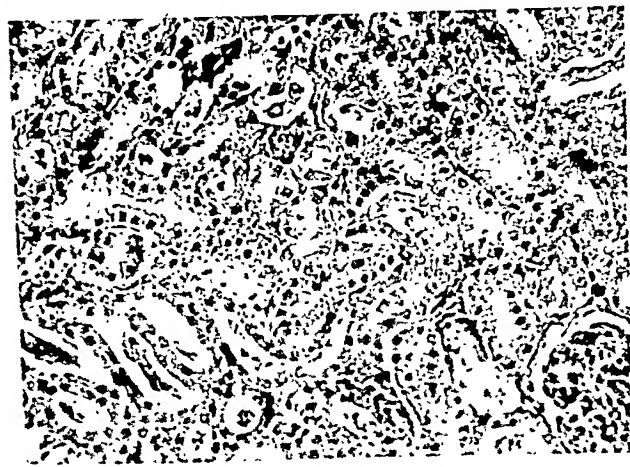
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Baxter

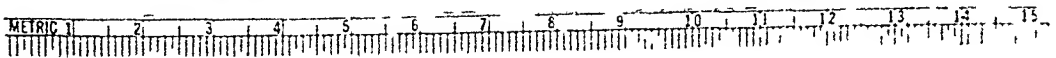
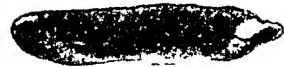
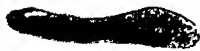
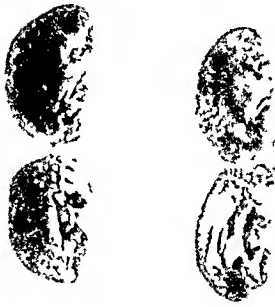
Hepatic and Renal Lesions Following Pyridine

PLATE 91

FIG. 5. The organs on the left side of the photograph are from the same rat as the section shown in Figure 4. The kidneys are comparatively large. This animal, like most of those on diet 1 plus pyridine, grew poorly, and the small size of the testes and heart was probably not a specific effect of the pyridine. On the right are shown the organs of a litter mate which received the same diet, plus 0.5 per cent of added methionine, for the same length of time. $\times 1$.

FIG. 6. Photograph of liver, kidneys, and spleen of a rat which died after 100 days on diet 3 plus 0.7 per cent pyridine citrate. The surfaces of the liver are finely granular. A section of this liver is shown in Figure 7. $\times 7/10$.

5



6



PLATE 92

FIG. 7. Section from the liver seen in Figure 6, showing cirrhosis with little fat. Extensive necrosis and regeneration have occurred, and again the characteristic structures of the portal triad were seen at the centers of many of the pseudo-lobules. Although the fibrous tissue was not abundant in most areas, it involved all of the lobules fairly uniformly. Hematoxylin and eosin stain. $\times 150$.

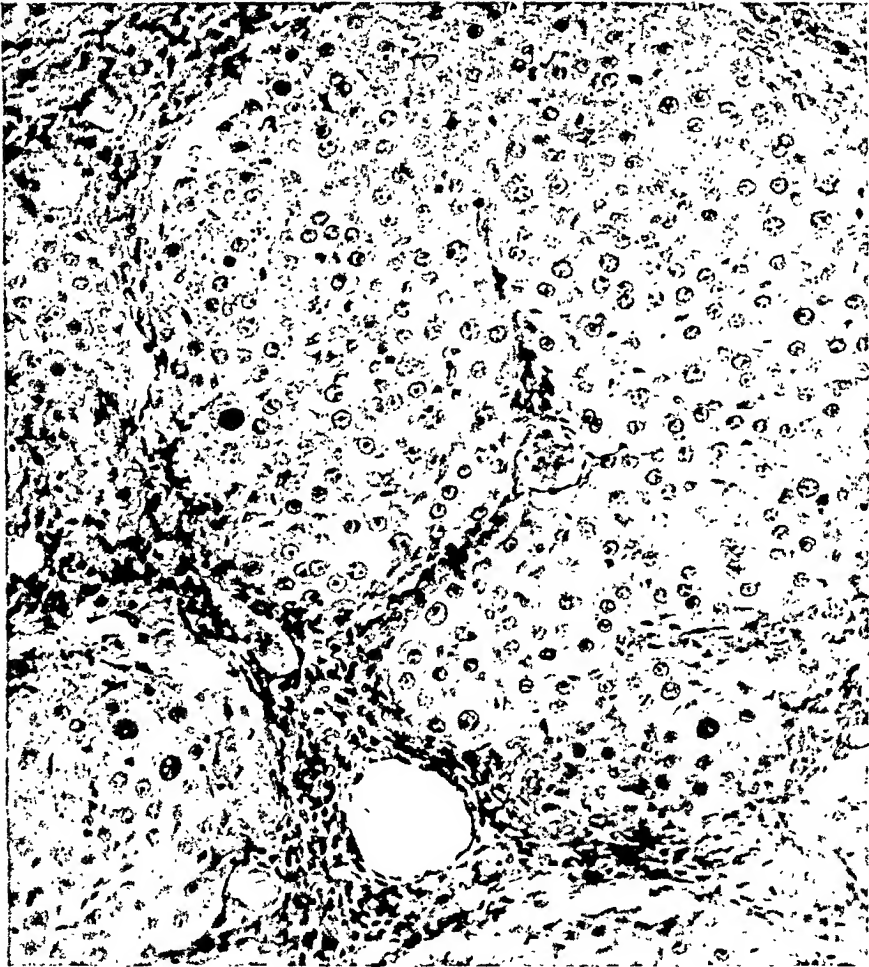
FIG. 8. Kidney from the same animal as Figure 7, showing chronic changes characterized by regeneration of epithelium and tubular dilatation. Hematoxylin and eosin stain. $\times 150$.

FIG. 9. Another area of the kidney shown in Figure 8. The original epithelial lining of the tubule apparently has been sloughed into the lumen, and the tubule is now lined by newly regenerated cells. The nuclei are closely placed, and vary in size, shape, and relative position in the cells. Hematoxylin and eosin stain. $\times 300$.

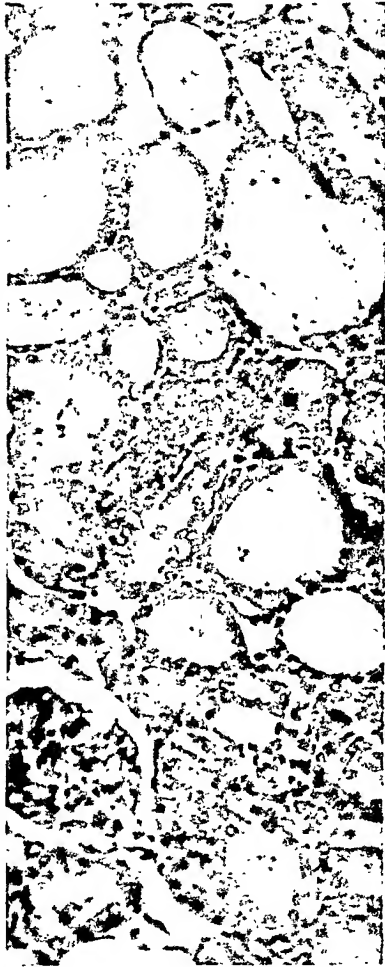
FIG. 10. One of the fairly numerous mitotic figures noted in the kidney from a rat on diet 3, with 0.34 per cent pyridine citrate for 2 months and 0.7 per cent pyridine citrate for an additional 2 months. Animal was found in shock and was autopsied. Hematoxylin and eosin stain. $\times 1000$.

FIG. 11. Nodule of liver cells from a rat which died after receiving diet 3 with 0.7 per cent pyridine citrate and choline *ad libitum* for 60 days. Most of the cells in this nodule were necrotic, without stainable nucleus or with only nuclear fragments. Various stages of calcification of the necrotic cells are evident. Hematoxylin and eosin stain. $\times 300$.

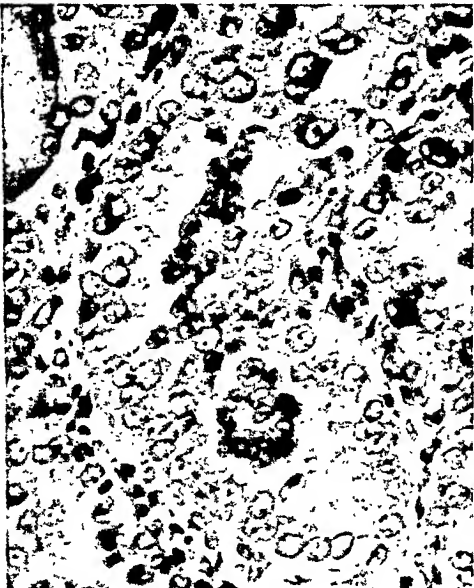
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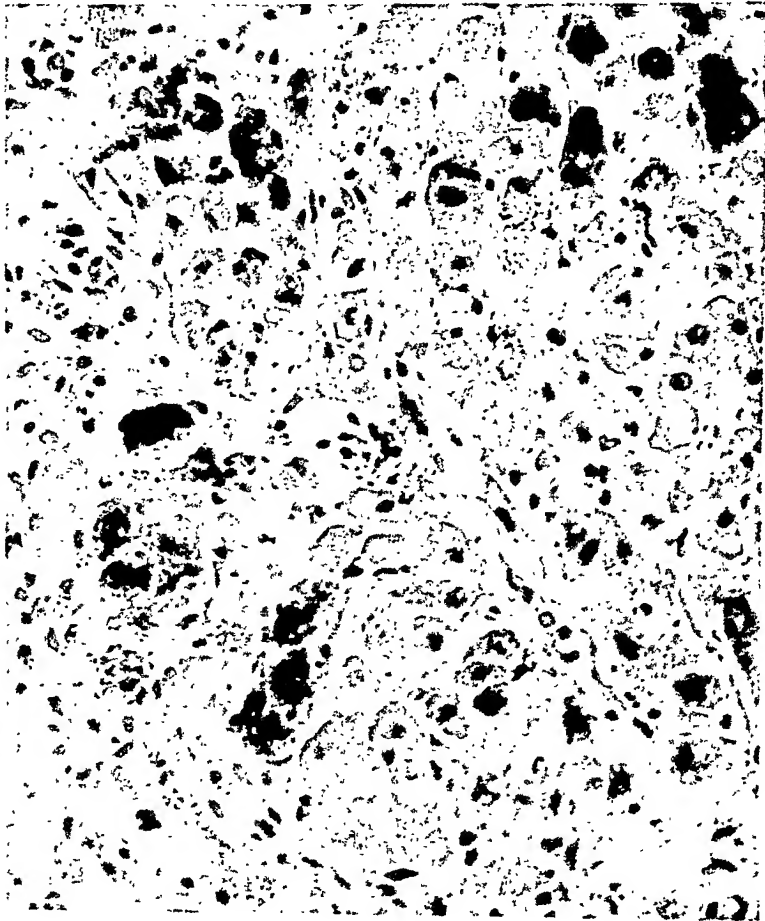
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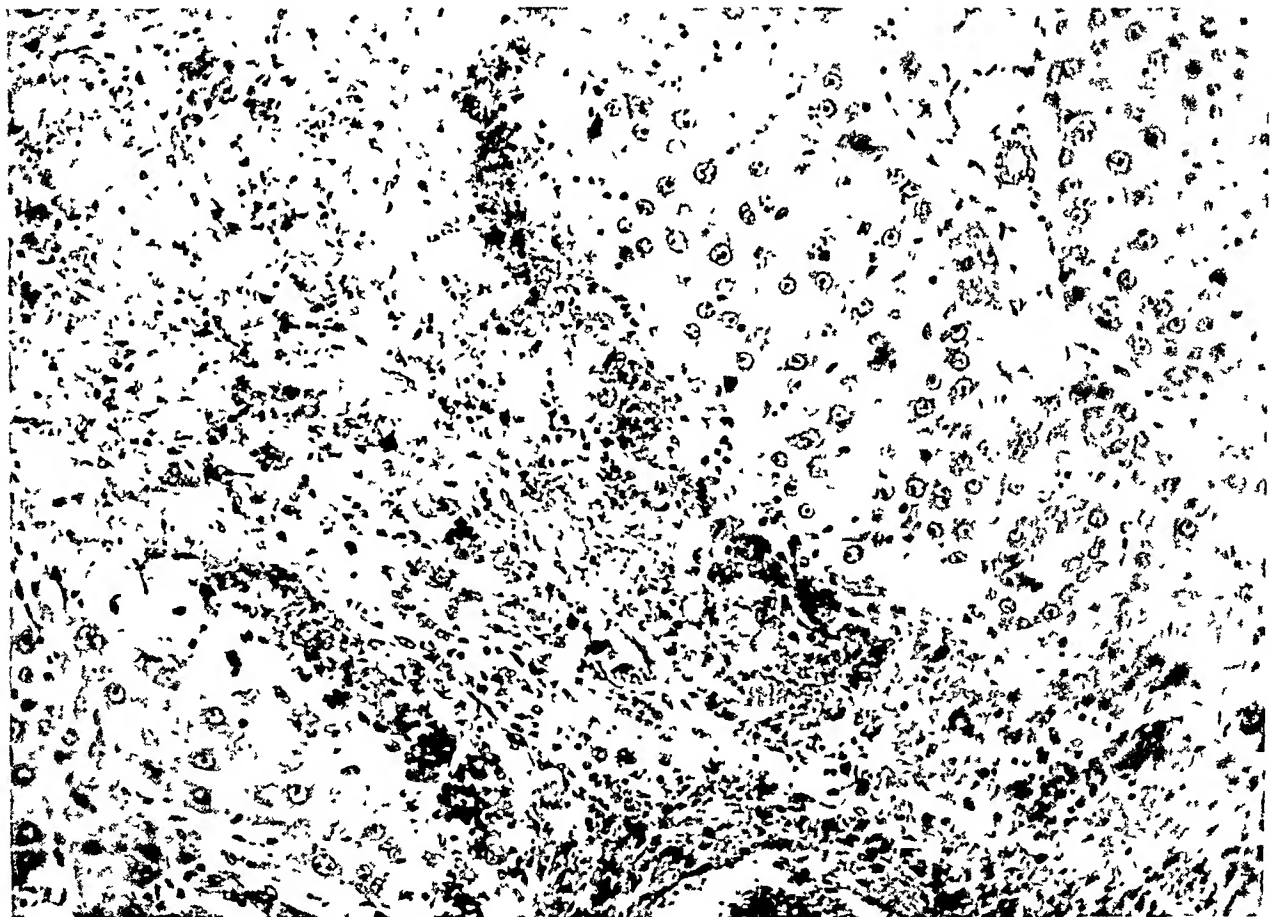


PLATE 93

FIG. 12. Liver from the same animal as Figure 10, showing the characteristic location and distribution of the calcified cells. They occurred chiefly at the edges of the old necrotic areas and closely surrounded the nodules of viable cells. Here again a bile duct and other structures of a portal triad are seen at the center of a nodule of liver cells. Hematoxylin and eosin stain. $\times 150$.

FIG. 13. A section of the same liver as in Figure 12, showing the calcium deposits stained black with silver. The preparation was made by placing the section in a solution of silver nitrate and exposing it to ultraviolet light, according to the method of von Kossa. $\times 150$.

12



13

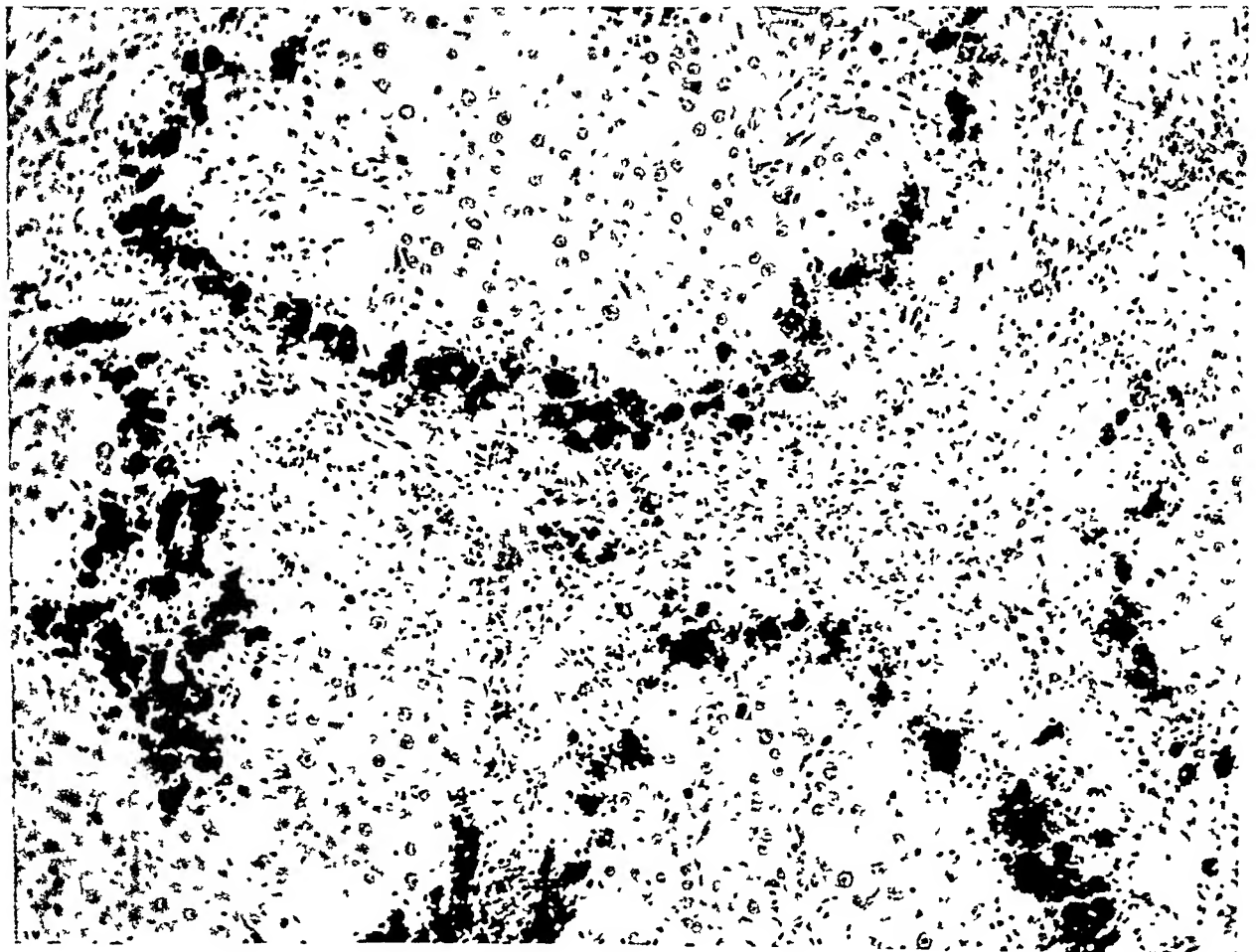


PLATE 94

FIG. 14. A section of the same liver as used for Figures 12 and 13, showing the transition, at the edge of an old area of necrosis, from the normal liver cells at the top of the section to the eosinophilic remains of cells at the bottom. The cells in the intermediate zone are calcified. Hematoxylin and eosin stain. $\times 700$.

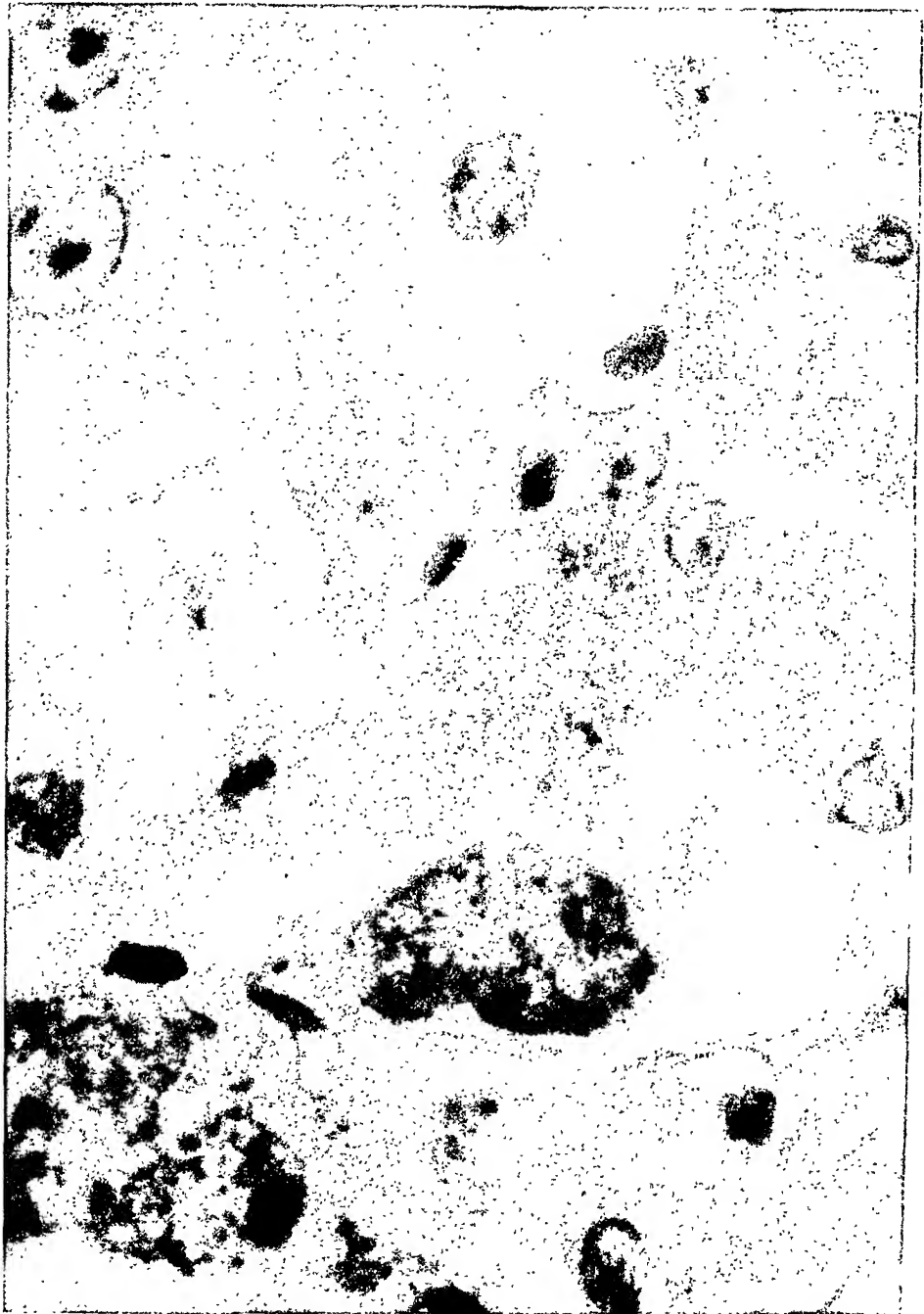
FIG. 15. Another area of the same section as for Figure 14, showing the various forms of cellular degeneration observed in passing from the fairly normal cells of a hepatic lobule at the upper left to the hyalinized remains at the lower right. The early stages of calcium deposition are well shown. Some of the calcium bodies were in focus and are seen as smoothly oval globules of varying sizes. The bodies seemed to be darker at the periphery than at the centers in many cases. Hematoxylin and eosin stain. $\times 1000$.

FIG. 16. Section showing the edge of a large tumor-like nodule of regenerative hepatic cells from the same liver as Figure 11. The cells are of large size. The dark-staining liver cells embedded in the fibrous tissue are calcified. Hematoxylin and eosin stain. $\times 100$.

14



15



16



BILIARY XANTHOMATOSIS (XANTHOMATOUS BILIARY CIRRHOSIS)*

H. EDWARD MACMAHON, M.D.

*(From the Department of Pathology and Bacteriology, Tufts College Medical School,
Boston 15, Mass.)*

Ten years ago Thannhauser and Magendantz¹ referred to a peculiar histologic change in the liver which they called "xanthomatous biliary cirrhosis." They distinguished this entity clinically and anatomically from other types of biliary cirrhosis associated with simple obstruction of the common bile duct. The specific histologic lesion, and the one on which the term was based, consisted of xanthoma cells and increased fibrous tissue in the walls of the intrahepatic system of bile ducts. This xanthomatous deposition in the liver, like xanthomata in other parts of the body, was considered to be a local manifestation of a hereditary and systemic disorder which they called essential hypercholesteremic xanthomatosis. The term "xanthomatous biliary cirrhosis" was used at the same time to designate a clinical syndrome in which xanthomatous change in the liver was but one of its most conspicuous features. This syndrome was characterized by chronic jaundice, an enlarged liver, hypercholesteremia, and, above all, xanthomatosis. It is important at this point to emphasize the double meaning that was assigned to "xanthomatous biliary cirrhosis," and it must be clearly understood that the changes throughout the liver were considered to be but one component of a systemic disease and not the cause of the syndrome. This has been confusing for it is always ambiguous to use the same term to denote a specific anatomic lesion, and to designate a clinical syndrome when that particular anatomic lesion is not regarded as the cause, but merely an integral part of the syndrome.

MATERIAL FOR STUDY

Through the interest and cooperation of Dr. Thannhauser, it has been my privilege to study adequate biopsy sections from the livers of 4 patients whom he selected as showing the signs and symptoms of the syndrome "xanthomatous biliary cirrhosis." Each of the 4 patients had been jaundiced for months and each had an enlarged and palpable liver. The cholesterol levels of the blood were above normal and each

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patient manifested the peculiar xanthomatous changes on the hands and body which are so characteristic of this disorder. At the time of biopsy, the extrahepatic biliary tract was normal. Two of these patients subsequently died at intervals of about 1 and 2 years after the original specimens were obtained. This enabled a follow-up study to be made and afforded the opportunity to compare early and late lesions.

BIOPSY FINDINGS

It was naturally anticipated that collections of xanthoma cells would be found in the walls of the bile ducts since this had been described as the specific histologic lesion peculiar to this clinical disorder. The findings in all four specimens were similar, but they were very disappointing, for in not one of the sections were xanthoma cells found in the walls of the bile ducts. In other words, there was no evidence in any of the biopsies of a lesion that one could call "xanthomatous biliary cirrhosis." Instead, there was a chronic proliferative and exudative inflammatory reaction in the portal areas which was most concentrated about the junctional ducts (canals of Hering) and terminal bile ducts (interlobular bile ducts or cholangioles) at the periphery of the lobules (Fig. 1). The portal areas were larger, broader, and longer than usual and often fused with one another to form rings of perilobular fibrosis (Fig. 2). Inflammatory granulation tissue extended into the periphery of the lobules. It blocked canaliculi, destroyed liver cells, and collapsed many sinuses (Figs. 3 and 4).

The larger bile ducts were patent and empty. The small interlobular bile ducts were very difficult to find and in many of the portal areas there were none. The junctional ducts, which in a normal liver are inconspicuous, were numerous, elongated, branching, and tortuous. Some were dilated and filled with bile, others were collapsed and empty. None of the ducts contained leukocytes, although several types of inflammatory cells, including lymphocytes, plasma cells, histiocytes, neutrophils, and occasionally eosinophils, richly infiltrated the surrounding granulation tissue.

The lobular pattern of the liver was well preserved and most central veins bore a normal relationship to the surrounding liver parenchyma. Large and sometimes lamellated bile casts were fairly numerous within the lobules (Fig. 5). These distended the canaliculi and often damaged the bordering liver cells. For the most part the liver cells were healthy; a few contained fat droplets; and some lying in the field of inflammatory reaction about the portal areas showed degeneration and necrosis. There were few mitotic figures and the presence of closely

packed clusters of small cells near the periphery of the lobules suggested a moderate degree of liver cell regeneration (Fig. 6).

In one of the four specimens the damage to the parenchyma was greater than in the others. In this there were fields in which the inflammatory reaction had cut deeply into the centers of the lobules (Fig. 7). In this way central veins became linked with portal areas and large and small islands of liver cells became isolated from the body of the parent lobules. These islands, in turn, seemed to melt away to become replaced by inflammatory granulation tissue (Fig. 8). In one area, an entire lobule had been destroyed and was now substituted by fibrous tissue.

The one fundamental histologic finding common to all four specimens was a chronic inflammatory reaction in the interstitial portal areas. It began as a chronic pericholangiolitis and spread into the peripheral zones of the adjacent lobules.

AUTOPSY FINDINGS

To understand cirrhosis it is necessary to know it in all its stages, and the opportunity to compare sections from the livers of patients during life with sections obtained months and years later in the course of autopsies carries with it a definite responsibility, particularly when the liver is the seat of a little understood and very debatable disease process.

Grossly, the livers of the 2 patients who were autopsied were large, firm, and cobbled. They weighed 2600 and 2800 gm., respectively. On section the texture was coarse and the parenchyma bile-stained. There were no depositions of cholesterol in the mucosa of the gallbladder or in any of the bile ducts. There were no concretions and, finally, there was no suggestion of any type of extrahepatic biliary obstruction.

The histologic features were much more complex and confusing than in the earlier material. In a few areas the central veins, central zones, and mid-zones were still recognizable, but for the most part the normal lobular pattern was now severely distorted. Bands of fibrous tissue divided the parenchyma into irregular and uneven nodules. The portal areas were composed of wide and communicating bands of connective tissue that extended deeply into the parenchyma and occasionally replaced whole lobules (Fig. 9). The parenchyma was composed of irregular cords of liver cells separated by edema fluid, dilated sinuses, and fibrous tissue. Here and there, the Kupffer cells were large, swollen, and filled with lipids, and nests of these cells occasionally distended the sinuses and compressed the adjacent trabeculae (Fig. 10). There was

much bile stasis, with bile in the canaliculi, liver cells, and sinuses. The larger bile ducts were collapsed and empty. The terminal ducts were inconspicuous and embedded in fibrous tissue. In none of the ducts was there either an inflammatory exudate or any evidence of xanthomatosis.

The large size of the liver, the extensive fibrosis, the fragmentation of some lobules and the total loss of others, the nodules of regenerated liver tissue, the compression and interruption of liver cords by fibrous tissue, the bile stasis, the intralobular lipid deposition, and, finally, the presence of a still active chronic inflammatory reaction in portions of the interstitial tissue all combined at this late stage to form a very confusing histologic picture. If such a damaged liver were to be seen for the first time, without a previous biopsy, its pathogenesis would be difficult to unravel and its classification would be uncertain.

COMMENT

Because the etiology of this disease of the liver is still unsettled, the descriptive term "pericholangiolitic biliary cirrhosis" is suggested. This emphasizes the inflammatory nature of the process and at the same time clearly denotes the exact site of the earliest lesion. It resembles other types of biliary cirrhosis such as the obstructive and the cholangiolitic, but from each of these it may, particularly in the early stages, be readily distinguished. There now seems to be little justification for referring to this change in the liver as xanthomatous biliary cirrhosis because this type of cirrhosis may exist in the absence of xanthomatosis.

"Pericholangiolitic biliary cirrhosis" with its destruction of terminal bile ducts and liver cells, and its proliferation of granulation tissue and subsequent fibrosis explains the retention and regurgitation of bile and the appearance clinically of jaundice. It will also account for the enlargement of the liver. Could it alone be responsible for the great increase of cholesterol and for the appearance of xanthomatosis? This is a debatable question. It must be admitted that bile stasis alone is rarely the cause of xanthomatosis. Yet, in this small collection of 4 patients and in all other cases that have been reported (Table I), long-standing jaundice consistently has been the earliest symptom. This implies that an interference in the normal secretion and elimination of bile, although perhaps not the only factor, is at least essential. In this respect, it is of interest to note that the syndrome is found almost exclusively in females of about 40 years of age. This suggests that constitutional factors, particularly those associated with age and sex,

TABLE I
Cases That Have Shown the Clinical Syndrome of "Xanthomatous Biliary Cirrhosis" in which the Liver Was Examined

	Date	Author	Sex	Age	Examination	Diagnosis
1	1869	Murchison ²	M	41	Autopsy	Cirrhosis
2	1873	Fagge ³	F	Adult	Autopsy	Cirrhosis
3	1873	Moxon ⁴	M	32	Autopsy	Stricture of bile duct*
4	1873	Pye Smith ⁵	F	49	Autopsy	Gallstone obstruction*
5	1905	Futcher ⁶	F	39	Laparotomy	Gallstone obstruction*
6	1905	Futcher ⁶	F	39	Autopsy	Gallstone obstruction*
7	1905	Futcher ⁶	F	42	Laparotomy	Hypertrophic cirrhosis
8	1908	Pinkus and Pick ⁷	F	Adult	Autopsy	Hypertrophic biliary cirrhosis
9	1909	Posner ⁸	F	37	Laparotomy	Cirrhosis
10	1911	Chvostek ⁹	F	47	Autopsy	Hypertrophic biliary cirrhosis
11	1924	Weidman and Freeman ¹⁰	M	6	Autopsy	Stricture of common duct*
12	1928	Dyke ¹¹	F	44	Autopsy	Hypertrophic biliary cirrhosis
13	1938	Thannhauser ¹	F	35	Autopsy	Xanthomatous biliary cirrhosis
14	1938	Thannhauser ¹	F	32	Laparotomy	Xanthomatous biliary cirrhosis
15	1938	Montgomery ¹²	F	29	Laparotomy	Postoperative stricture*
16	1938	Montgomery ¹²	F	43	Laparotomy	Postoperative stricture*
17	1938	Montgomery ¹²	F	48	Laparotomy	Postoperative stricture*
18	1938	Montgomery ¹²	F	37	Laparotomy	Postoperative stricture*
19	1944	Eusterman and Montgomery ¹³	F	48	Autopsy	Cirrhosis
20	1945	Hoffbauer, Evans, and Watson ¹⁴	F	62	Autopsy	Gallstone obstruction*
21	1947	Thannhauser, MacMahon	F	44	Biopsy	Pericholangiolitic biliary cirrhosis
22	1947	Thannhauser, MacMahon	F	38	Biopsy	Pericholangiolitic biliary cirrhosis
23	1947	Thannhauser, MacMahon	F	46	Biopsy	Pericholangiolitic biliary cirrhosis
24	1947	Thannhauser, MacMahon	F	43	Biopsy	Pericholangiolitic biliary cirrhosis

* Cases showing extrahepatic biliary obstruction (10 of 24).

may be important. It would appear that once this type of biliary cirrhosis has become established, it could lead, under certain conditions, to the complete clinical syndrome.

Another question that may be asked is this: Is "pericholangiolitic biliary cirrhosis" the only type of liver disease that may lead to, or be associated with, this clinical syndrome? If one includes all degrees of this clinical disorder the answer is no, since almost half of the cases reported in the literature have been secondary to some form of extra-hepatic biliary obstruction, and at least two types of biliary cirrhosis, namely, the obstructive and the cholangiolitic, may be associated with obstruction. It is obvious, then, that there is no basis for considering this syndrome as the manifestation of a single and specific disease of the liver, and, as a corollary, it becomes clear that there is no longer any justification for including this syndrome in the family of "hereditary essential hypercholesteremic xanthomatoses."

It will be misleading to continue to refer to the changes in the liver in this syndrome as xanthomatous biliary cirrhosis, and it may be equally confusing to retain this same name in referring to this peculiar clinical disorder. Therefore, it is suggested that this term be discontinued and that a new term for the syndrome should be selected. Because jaundice has been the earliest clinical symptom, and because xanthomatosis is the most striking feature of the syndrome, the name "biliary xanthomatosis" is suggested. It has been pointed out that the findings in the liver in this disorder may vary. If one could be certain in a particular case that the underlying lesion in the liver was "pericholangiolitic biliary cirrhosis," then the more specific name for the clinical picture could be "pericholangiolitic xanthomatosis." If, on the other hand, some form of extrahepatic biliary obstruction was found to be the primary lesion, the term "obstructive xanthomatosis" would be in order.

I wish to express my sincere appreciation to Dr. S. J. Thannhauser for his encouragement and patience in the preparation of this paper and for the use of this clinical material for publication.

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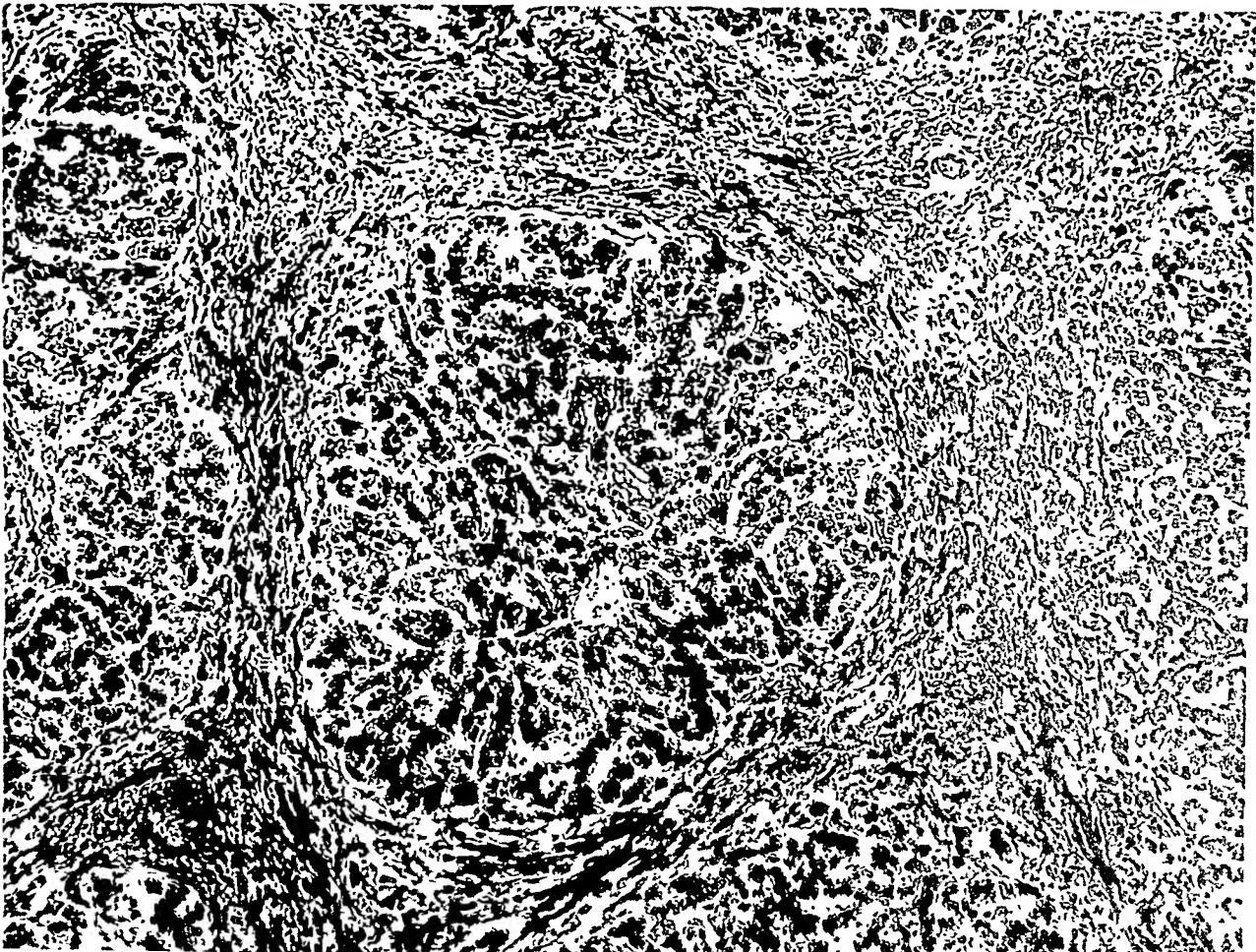
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 95

- FIG. 1. Liver. The center of the field is occupied by a single lobule of liver tissue that is sharply demarcated by a band of inflammatory granulation tissue. The central vein lies at about the center of the lobule. The sinuses, moderately dilated, bear a normal relationship to the cords of liver cells. The portal connective tissue contains small arteries and collapsed bile ducts, but neither veins nor lymphatics are recognizable. There is no increase of reticulum within the substance of the lobule. $\times 65$.
- FIG. 2. Liver. A wide and roughly T-shaped portal area bordered by the peripheral zones of three adjacent lobules occupies most of the field. The portal vein is collapsed, obliterated, and overgrown by inflammatory granulation tissue. There is a moderately rich infiltration with lymphocytes, histiocytes, a few plasma cells, eosinophils, and polymorphonuclear leukocytes. At several points the inflammatory granulation tissue extends superficially into the adjoining lobules. There are no recognizable interlobular bile ducts in the whole area. The dilated, but intact, central vein in one of the three lobules stands out in striking contrast to the changes in the portal areas and peripheral zones of the lobules. $\times 85$.

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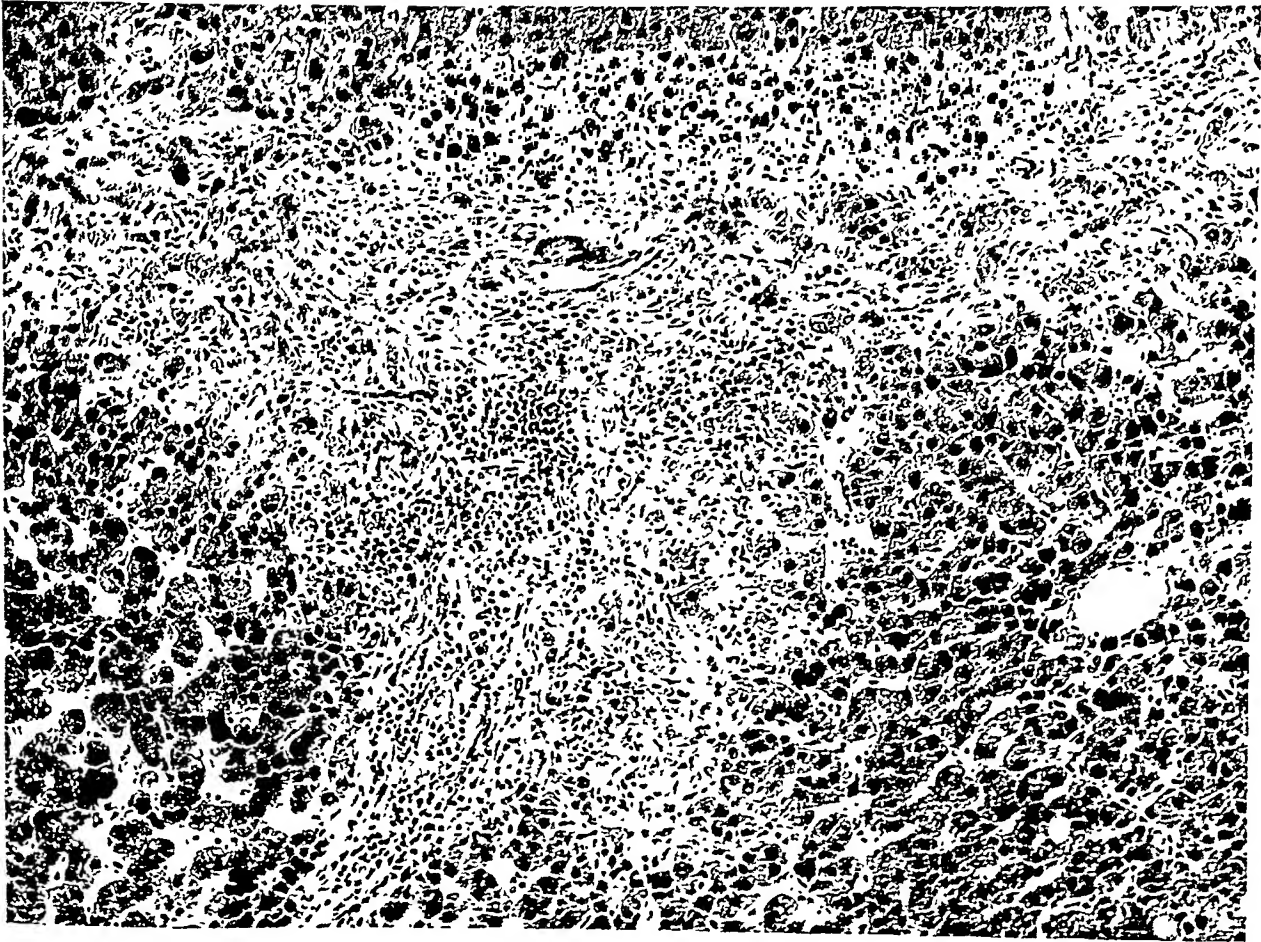
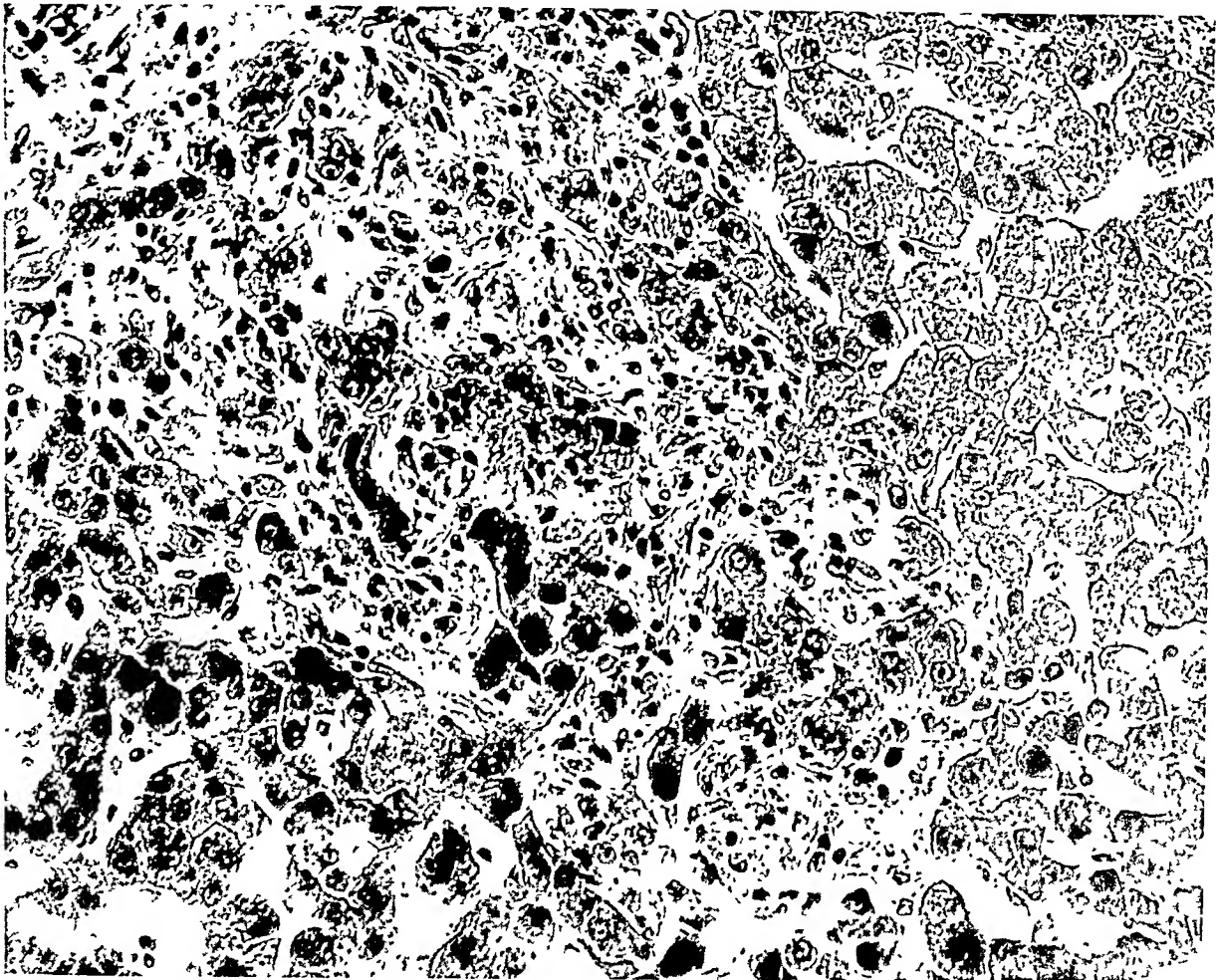


PLATE 96

FIG. 3. Liver. This field was selected to show a broad, tongue-like expansion of inflammatory granulation tissue cutting into the outer portion of a lobule. Cords and nests of liver cells are completely surrounded by this edematous cellular inflammatory tissue. Sinuses are collapsed or overgrown and in this area are no longer visible. $\times 170$.

FIG. 4. Liver, showing a small field just at the junction of portal connective tissue and liver lobule. This area was selected to show the extension of inflammatory granulation tissue along a sinus into the periphery of a lobule. Parallel cords of liver cells are separated and compressed. The sinuses are obliterated in some areas and compressed in others. There are no interlobular bile ducts in this area. $\times 260$.

3



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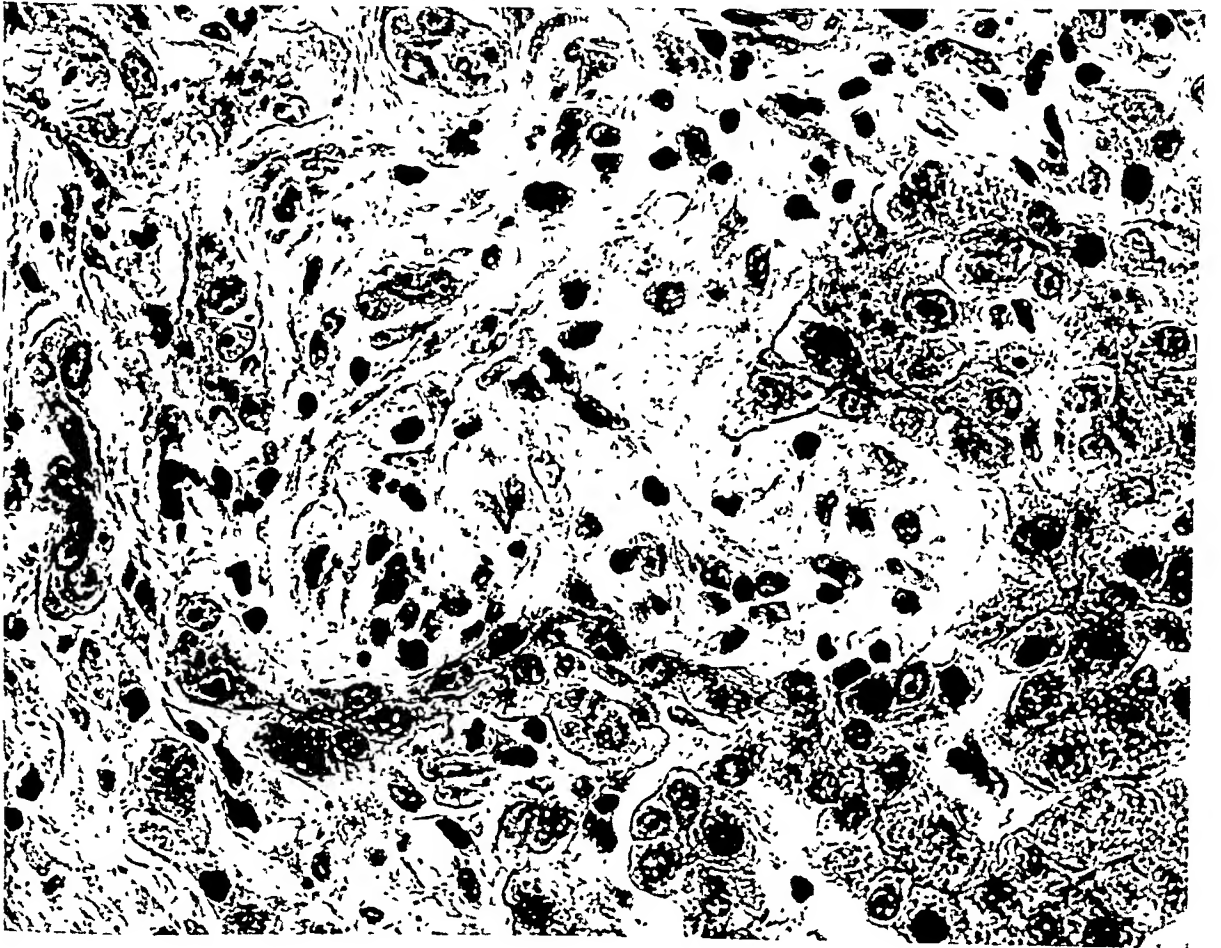
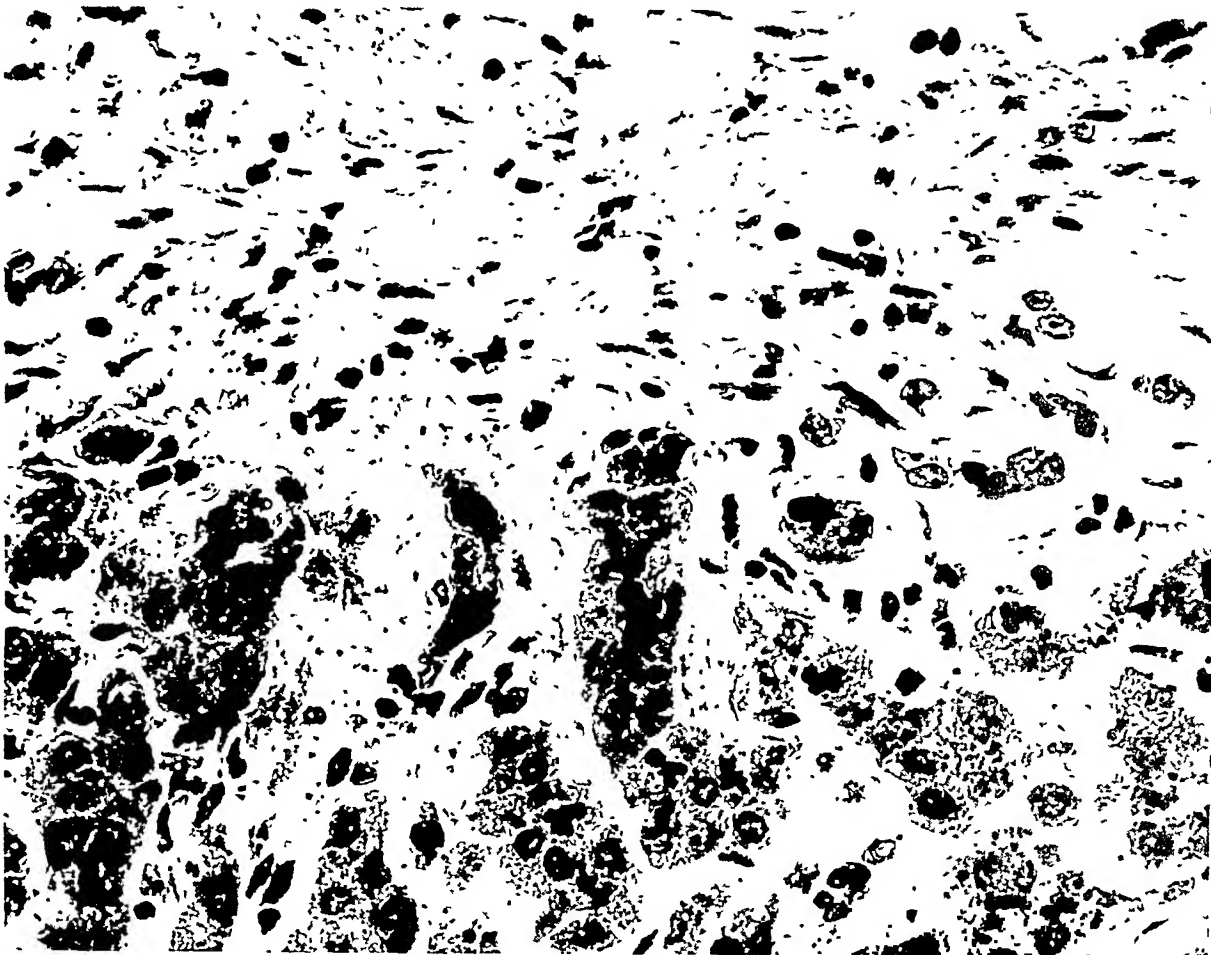


PLATE 97

FIG. 5. Liver. This field was selected to include the outer margin of the peripheral zone of a lobule and the adjoining and very much thickened portal connective tissue. A sharp line separates the two. The most significant finding is the bile stasis with large casts of inspissated bile distending bile canaliculi. The portal area is edematous and is composed of fibrous tissue containing few cords of liver cells. In this field there are no interlobular bile ducts. $\times 265$.

FIG. 6. Liver. This field was selected to show a liver cell in mitosis. The dividing nucleus lies in a compact nest of liver cells in the peripheral zone of the lobule. The involved cell is somewhat larger than the adjoining cells touching it and the chromatin of the dividing nucleus is about equally divided. One-half of the chromatin is suspended in a spindle which is clearly visible toward one end of the cell. $\times 350$.

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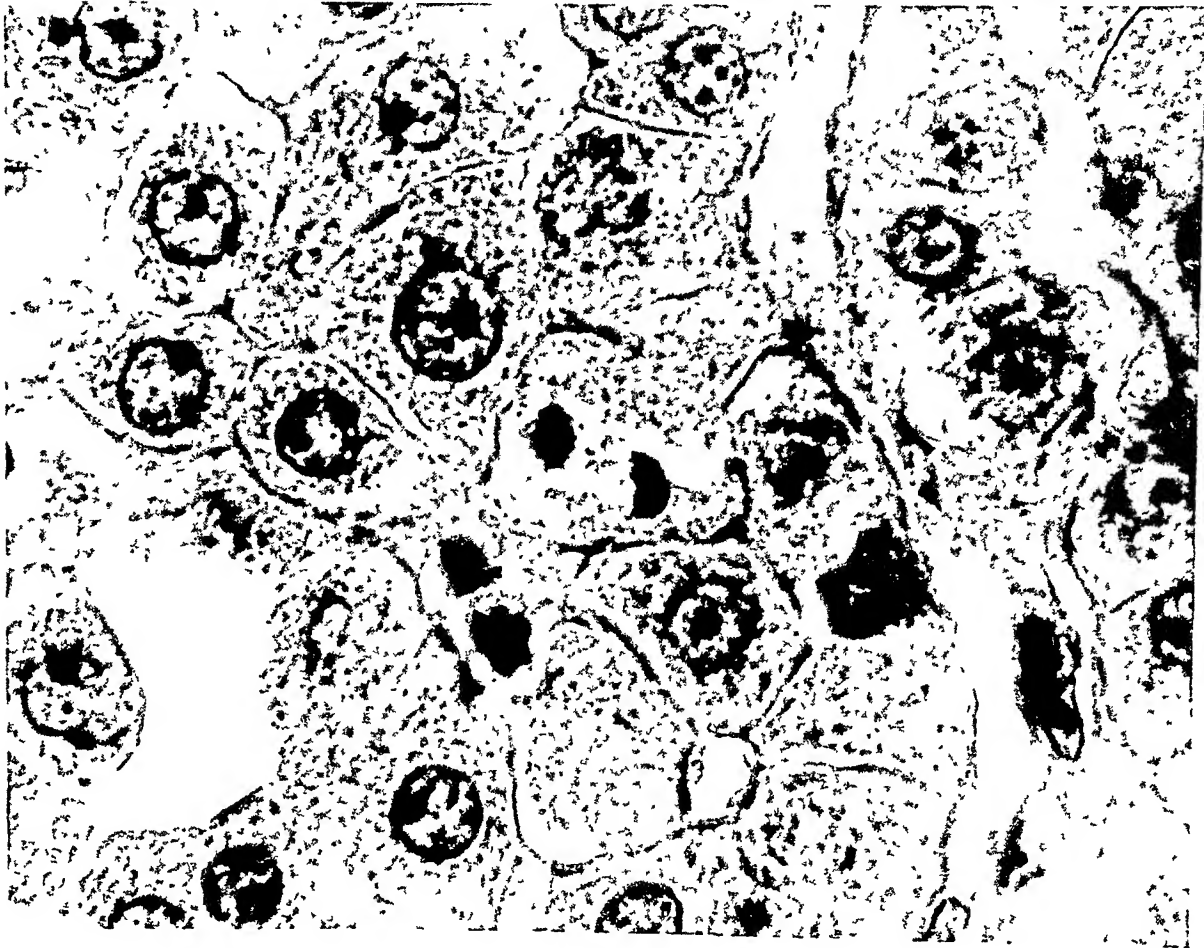
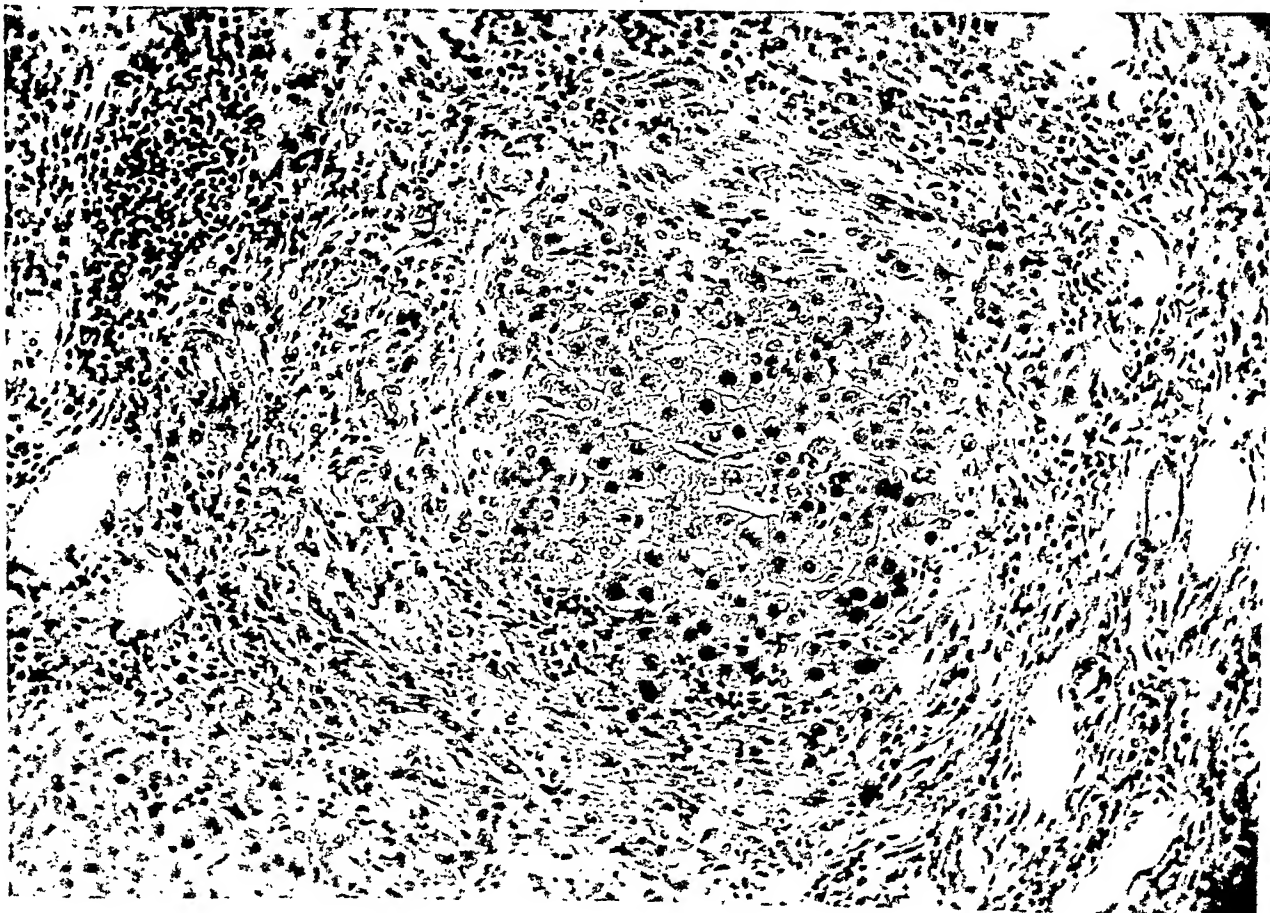
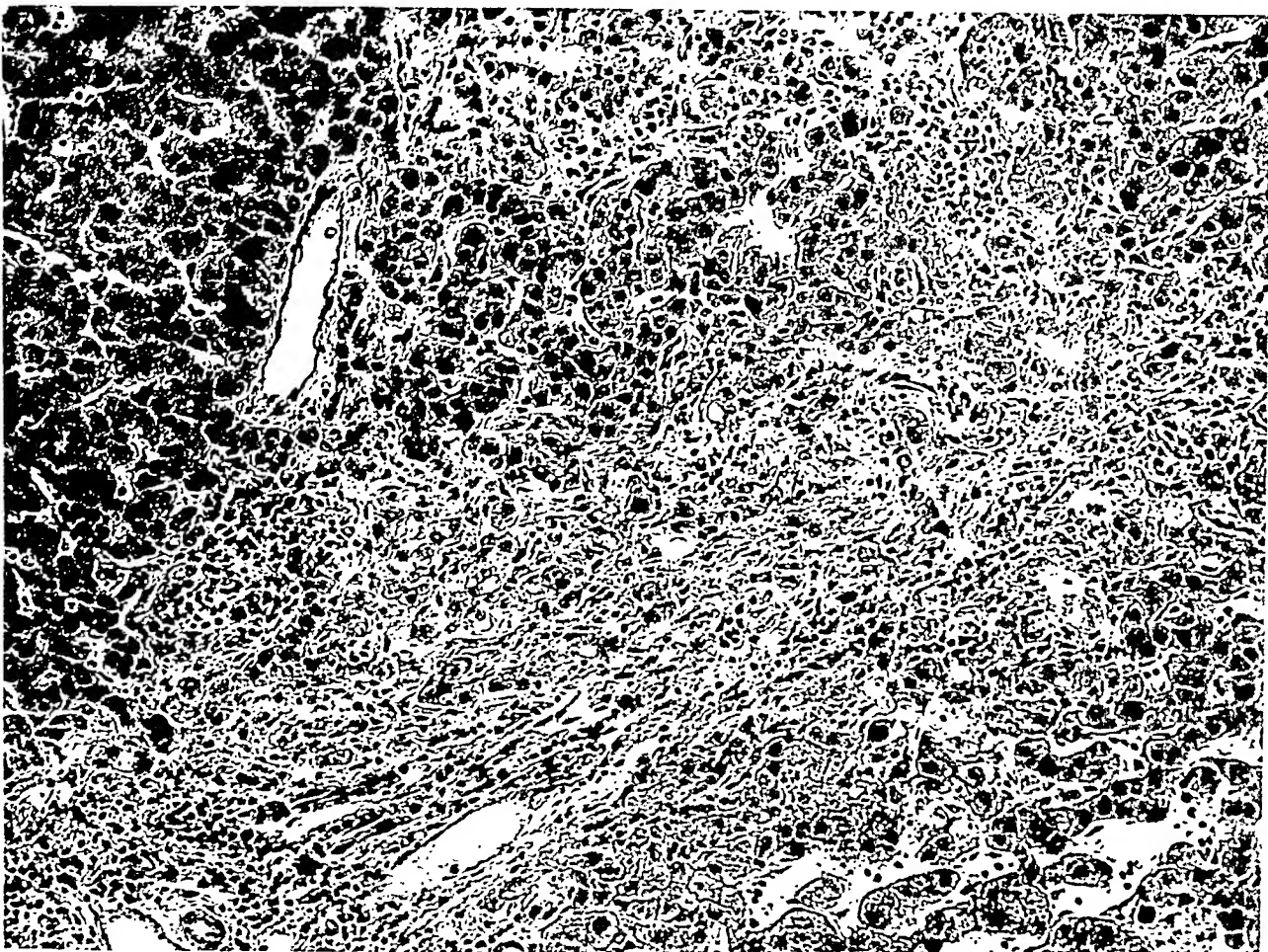


PLATE 98

FIG. 7. Liver. This field was selected to show encroachment on the lobule by inflammatory granulation tissue. The center of the field is occupied by a nest of compressed liver cells which is bordered on all sides except one by granulation tissue. One surface is bordered by a dilated central vein. Beginning in the portal area, the granulation tissue extends outward along the perisinusoidal spaces as far as the central vein. Many liver cells of the peripheral zone have disappeared. $\times 85$.

FIG. 8. Liver, showing a small compact nest of liver cells—all that remains of a lobule. It is completely surrounded by granulation tissue growing in from the surrounding portal areas. In the area of inflammation, all liver cells have disappeared. $\times 130$.



MacMahon

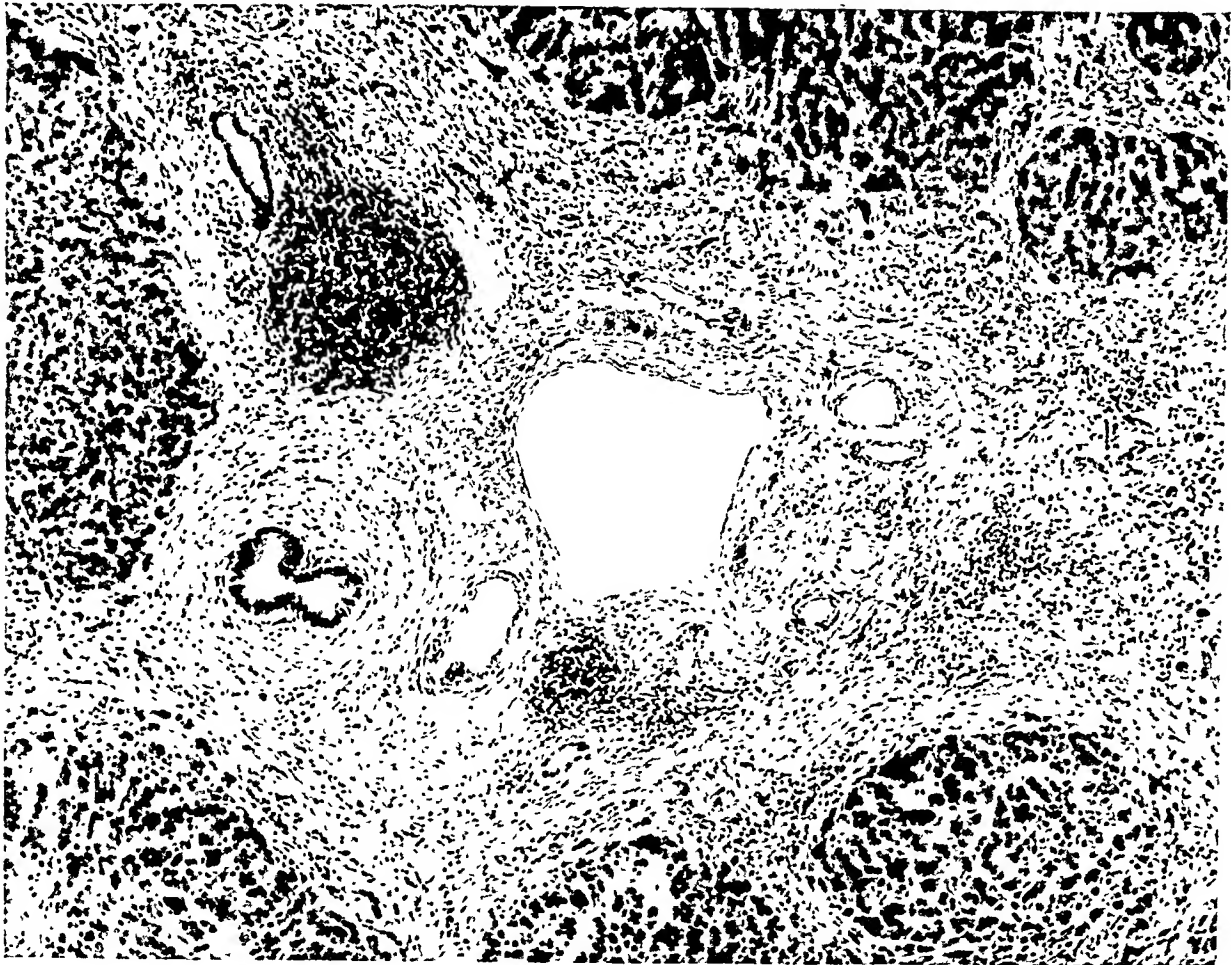
Biliary Xanthomatosis

PLATE 99

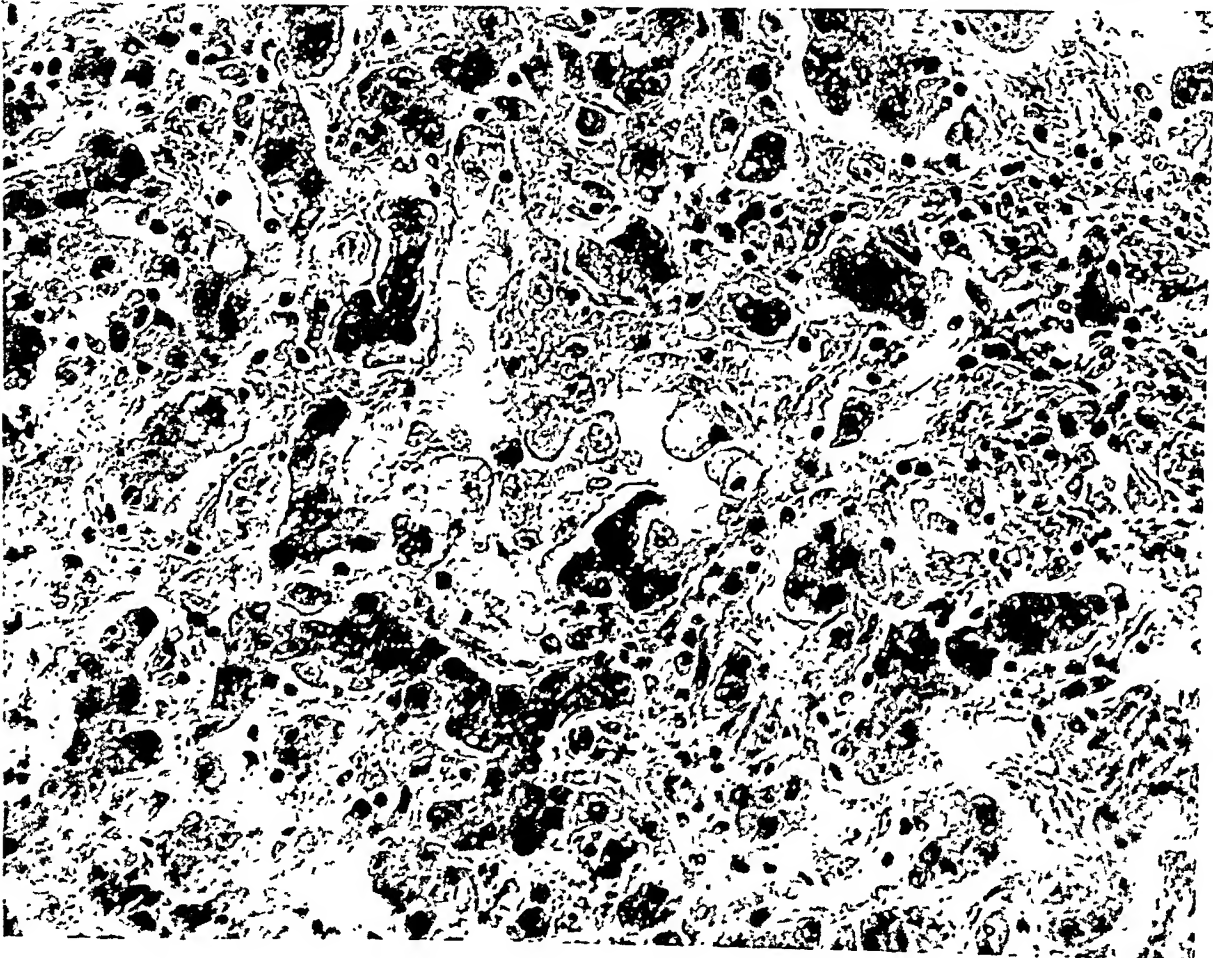
FIG. 9. Liver. The center of the field is occupied by a medium-sized portal area. The periphery is represented by portions of the adjacent lobules. The portal vein and its branches are dilated, as are the arteries. The medium-sized bile ducts are empty. There is a great increase in fibrous tissue expanding the entire portal area. There are nests of lymphocytes and narrow and compressed cords of liver cells. A fairly sharp line of demarcation separates fibrous tissue from the parenchyma of the adjoining lobules. $\times 45$.

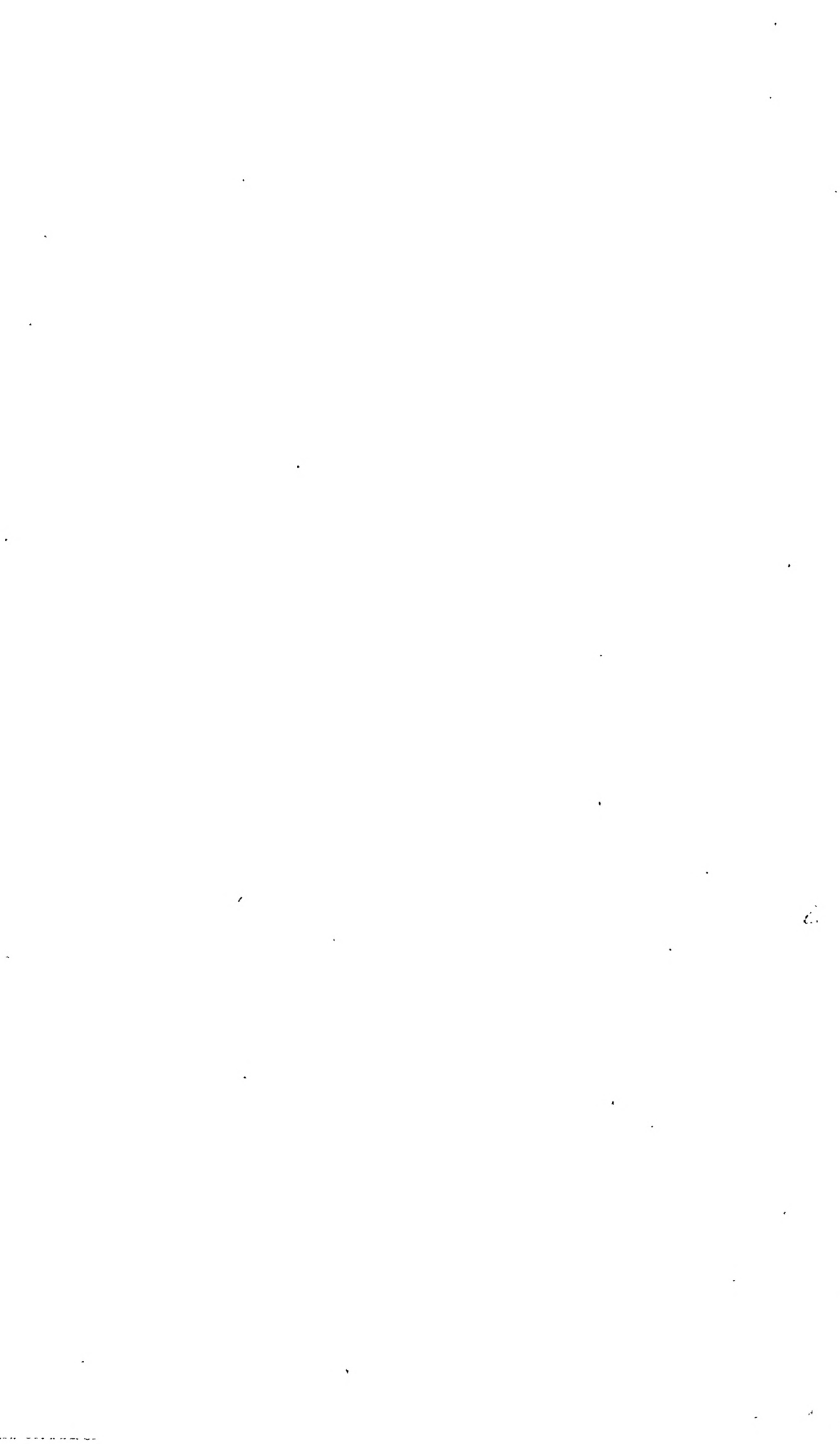
FIG. 10. Liver. This field was selected from the peripheral zone of a lobule to show collections of lipid-laden endothelial cells within the confines of the lobule. These cells lie in distended sinuses. The cords of liver cells are interrupted, compressed, and distorted. No bile ducts are visible in this area and no xanthoma cells are visible in the adjacent portal connective tissue. $\times 260$.

9



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CYTOLOGIC STUDIES WITH THE PHASE MICROSCOPE

I. THE FORMATION OF "BLISTERS" ON CELLS IN SUSPENSION (PODOCYTOSIS), WITH OBSERVATIONS ON THE NATURE OF THE CELLULAR MEMBRANE *

HANS U. ZOLLINGER, M.D.†

(From the Department of Pathology of Cornell University Medical College, and the New York Hospital, New York 21, N.Y.)

In a study of various tissue cells by means of the phase microscope (PM), a curious formation of "blisters" has been observed on several types of cells in suspension. The circumstances under which this phenomenon has been observed will be given in the present paper, along with certain implications which the findings may have in relation to the nature of the membranes surrounding cells.

THE PHASE MICROSCOPE

The PM equipment, first described by Zernike,¹ makes use of the following principle: If two lightbeams A and B (Text-Fig. 1) of the same wave length (λ) pass through two very thin glass plates, Ga and Gb, of the same thickness and refractive index, their original wave length becomes shorter because the refractive index of glass is higher than that of air. If the two beams enter the glass plates in the same phase of oscillation; they will leave it, and re-enter the air, still in parallel oscillations (A_1 and B_1). A third glass plate (Gc) of the same thickness, but darker and, therefore, absorbing a considerable amount of light, changes the amplitude c of a third lightbeam C into c_1 , whereas the amplitudes of A and B will not be changed theoretically by the plates Ga and Gb, because these plates absorb no light. The effect of lightbeam A_1 on the eye is the same as that of B_1 , while lightbeam C_1 appears less bright. A fourth beam D, going through a slightly thicker glass plate (Gd) will leave it in another phase of oscillation than the lightbeams A_1 , B_1 , and C_1 as seen in level X; there is a so-called "phase-difference" (PD) present. The same thing happens if light passes through small particles, which have a different index of refraction or a different thickness than the surrounding medium, as for instance in the case of mitochondria in protoplasm.

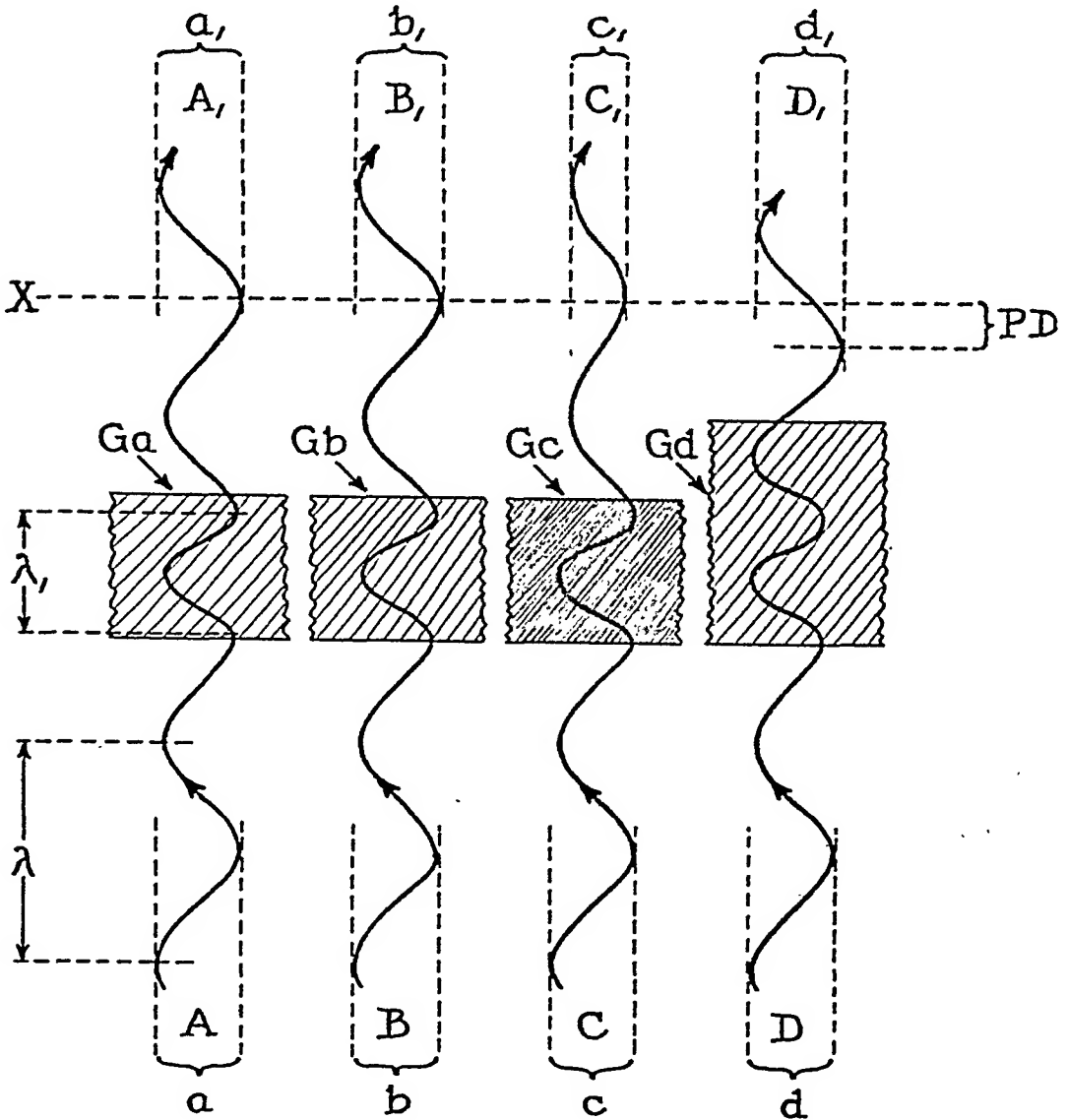
Unfortunately, the eye cannot recognize phase-differences in the ordinary microscope, but only differences in wave length (colors) and in intensity. The PM converts phase-differences into differences in amplitude by means of a "phase plate"; thus, it enables the human eye to perceive phase-differences as black and white contrasts. (For the theoretic explanation of the effect of the "phase plate" see Zernike,¹ Ganz,² and Bennett, Jupnik, Osterberg, and Richards.³) Therefore, the image can be observed with the PM without staining.

In practice, a number of intracellular constituents that are invisible or indistinct when studied by ordinary, darkfield, or ultraviolet microscopy have become readily visible in detail when examined with the

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† Fellow of the Swiss Foundation for Biological-Medical Fellowships.

PM. To provide a comparative illustration of the results obtained with an ordinary and a phase microscope, photographs were made of the cells of an adenocarcinoma of the stomach as viewed in various preparations with the two kinds of optical systems (Figs. 1 to 4). Furthermore, by means of the PM the investigator can follow consecutive intracellular changes for hours and even days in tissue cultures and thus obtain, so to speak, a "longitudinal section" through the whole



Text-Figure 1. Schematic drawing illustrating the effect of different glass plates on light beams which are in the same phase of oscillation. G_a and G_b are made of colorless glass of the same thickness as G_c (dark glass). G_d is made of colorless glass, but it is slightly thicker than the other three plates. This difference in thickness causes a phase-difference (PD) of the beam D .

course of a process, whereas the microscopic section shows but a "transverse section" through the process at the moment when the cell

was killed by fixation. On the other hand, the microscopist should be mindful of the fact that effective phase microscopy depends upon small differences in refraction or thickness between objects. Finally, the fact deserves emphasis that the resolving power of the PM theoretically cannot be higher than that of an ordinary microscope; nevertheless, it is possible to see elements with the PM which are invisible, owing to shrinkage caused by fixation and dehydration, in stained preparations examined with the ordinary microscope.

METHODS

The observations were made with a Zeiss instrument consisting of a centerable condenser with a ring-shaped diaphragm for each objective. The three objectives contained the phase plates. The photographs were taken with a 35 mm. "Alpa-Reflex" camera on Kodak Panatomic X film. As the cells floated freely in fluid, there was always a certain movement under the coverslip; hence photographs were rather difficult to take. Another difficulty was caused by the fact that the cells are three-dimensional. Thus, a certain level of a cell, actually in focus, may still appear indistinct because of the interference caused by elements in other levels.

Suspensions of normal cells of various types (kidney, liver, adrenal, stomach, and small intestine) procured from various animals (frog, rabbit, and mouse) were made by teasing, or washing, or scraping normal organs immediately after the animal was killed. Kidney cells of the frog proved especially suited to study with the PM, because they are not easily influenced by temperature, and they seem to survive the death of the animal for long periods (W. Lewis and McCoy⁴). In order to obtain suspensions of living tumor cells (Gardner's lymphosarcoma, C₃H sarcoma, and granulosa cell tumors of mice, V₂ and Brown-Pearce carcinomas of rabbits), pieces of the neoplastic tissues were removed with aseptic precautions immediately after the animal was killed, thoroughly freed from as much of the adjacent normal tissue as possible, dissected in small pieces, and passed through a 40 mesh Monel-metal sieve.

Physiologic saline (0.9 per cent) and buffered Ringer's solution containing 150 mg. per cent of glucose (BGR) were used as suspension media. Liver cells of the frog required 0.5 per cent of NaCl (Anitschkow⁵), whereas a concentration of 1.25 per cent of NaCl is considered by von Möllendorff⁶ to be physiologic for kidney cells. In order to watch the effects of various chemical agents, the cells were observed while the original suspension medium was replaced in the following

way: A drop of the chemical solution to be tested was placed on one edge of the coverslip and was drawn under the coverslip by means of filter paper placed at the opposite edge of the coverslip, which drew off the excess fluid. Of course, cells also were removed by this procedure, but there still remained a considerable number of cells in the microscopic field adhering either to the coverslip or the slide. During this replacement the cells were constantly observed with the high-power oil-immersion objective; the addition of a small amount of neutral red to the test solution facilitates the determination of the exact moment when the chemical reaches the cells under observation.

Each finding here reported has been confirmed by repeated observations in which different types of cells were used.

OBSERVATIONS

Various distinctive constituents of living unstained cells can be identified readily by means of the PM (Figs. 5 and 7). In undamaged cells, there usually is seen a distinct single-contoured, thin, cellular membrane, no matter whether the cell is isolated (Figs. 5 and 7) or located in the center of a small piece of tissue (Fig. 6). By means of this purely optical method it is impossible to distinguish between the plasma membrane and the extraneous cellular membrane (Chambers⁷).

The Formation of Blisters by Cells of Various Types in Suspensions

Cells in suspensions very often exhibit large "blebs" or "blisters" on their edges (Fig. 8). This phenomenon occurs in almost every kind of cell after the elements have remained in BGR or in isotonic salt solutions of other types for several minutes. In very fresh suspensions of the various cells studied, no blisters could be observed. The only cells that did not form blisters in these experiments were squamous cells from the mouth and tongue of man and frog. In the Shope papilloma only the basal cells showed blister formation.

The first signs of blister formation appear as early as 3 or 4 minutes after the suspension is made: small "cavities" arise in the protoplasm. They are usually adjacent to the cellular membrane, and later, as they grow in size, the cellular membrane bulges out until a blister is formed (Figs. 9 and 10). Then, the blister enlarges chiefly in width, thus detaching the adjacent membrane (Figs. 11 and 12), and finally the whole cytoplasm is surrounded by the contents of the blister, which separate the cytoplasm from the membrane (Fig. 13). Besides this type of blister formation, which may be called "diffuse," there occurs a "local" type (Fig. 14). In this, the base of the blister extends for

only a limited distance around the cell, and, after a time, the blister becomes more rounded. Since these two types can be observed in the same suspension, it does not seem that there is a fundamental difference between them.

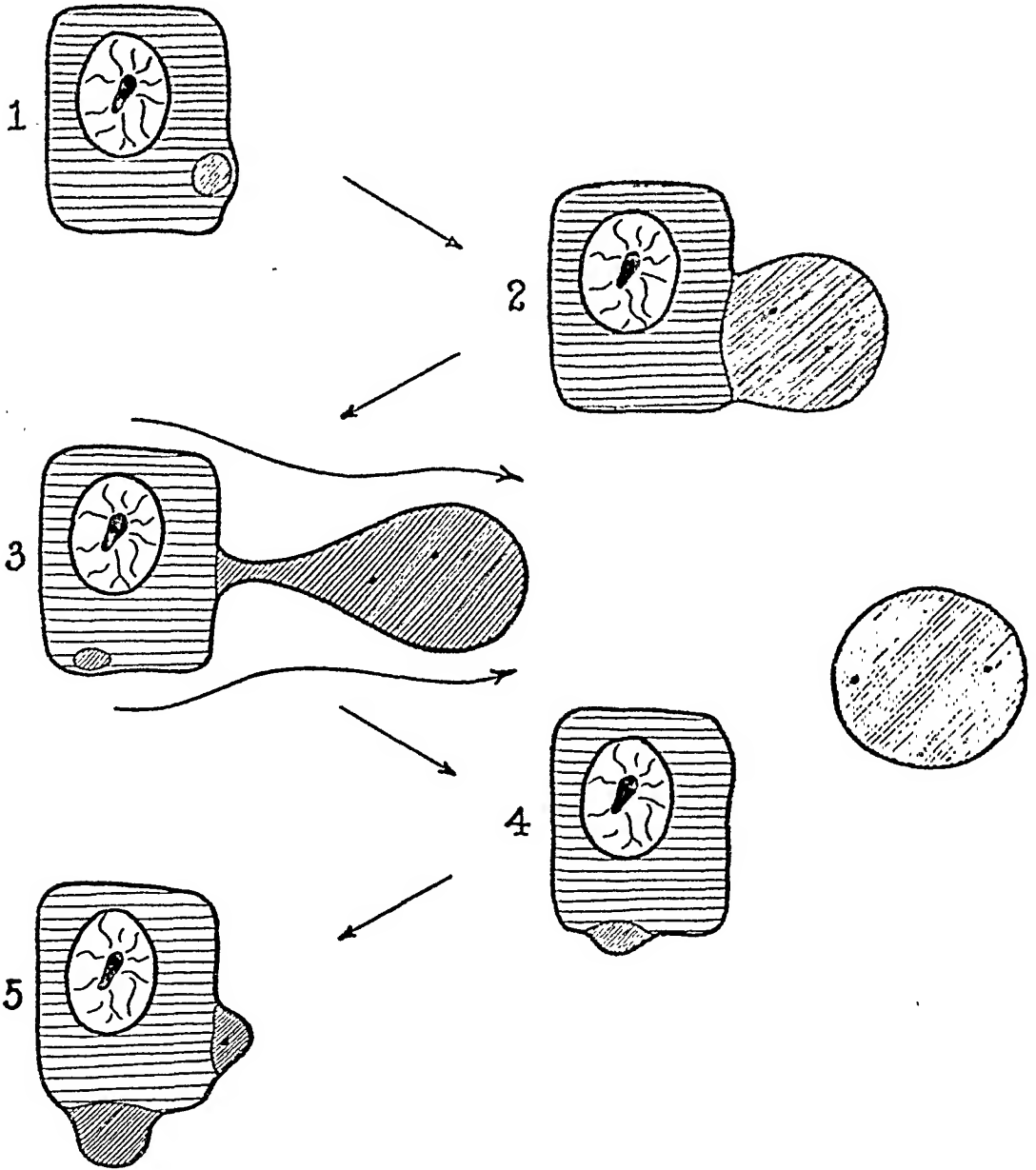
The blister formation in clumps of cells and tissue pieces is usually restricted to the free edges (Fig. 15); in the central region blisters have not been observed. In single cells the blister formation may begin on either side of the cell. Thus, there could never be observed a predilection for the inner face (apical pole) of single gland cells.

Figure 16 shows a very distinct boundary line between blister contents and protoplasm (see also Figs. 11 and 12). Occasionally, minute dark particles can be seen moving about in the blister contents. Their movement continues even if the blister wall is demolished; the suspension medium as a rule contains a number of such freely floating particles. Rarely, the blisters contain one or two round, brilliant, slowly moving granules of considerably larger size which show the same optical structure and the same reactions to chemical agents as do the brilliant granules in the protoplasm (see below). The blisters are filled with a homogenous material, which usually appears slightly darker in the PM than does the suspension medium (Figs. 11 and 12), and is not stained by neutral red in a concentration of 1:10,000.

A distinction can readily be made between blisters and vacuoles (Fig. 17). The latter are found in the protoplasm of "exhausted" cells, that is, cells which have been in the suspension medium for several hours, or have been heated (45° C. for 20 minutes). Vacuoles do not cause bulges in the cellular membrane, and are much brighter than the suspension fluid (Fig. 17). In ciliated epithelial cells, which contain vacuoles, the cilia never move and the nuclei soon disintegrate.

Although two blisters often touch, they usually remain separated by their walls (Fig. 15). This is also true for blisters which are exposed to pressure from one side. Figure 18 shows a cell presenting two blisters which have been moved slightly out of their original position by a fast current in the suspension medium. If the current becomes still more vigorous, the blisters are detached suddenly, and float freely in the suspension fluid. Text-Figure 2 shows schematically the entire process of blister detachment. Just before detachment, the blister becomes drop-shaped and the stalk appears more and more elongated until finally it is torn off. As soon as the blister is detached, it becomes spherical (Text-Fig. 2 and Fig. 19) and behaves like a rubber ball, its surface being momentarily indented if it bounces against an obstacle or a cell in the moving medium, immediately afterwards becoming

spherical again. Strong pressure on the coverslip divides free blisters into multiple small blisters. In detached blisters the black particles enlarge slowly, whereas the occasional brilliant granules do not change their size.



Text-Figure 2. Schematic drawing demonstrating the formation and the detachment of a blister. (1) Blister formation starts in the form of a submembraneous round space in the protoplasm. (2) A big blister with two granules has developed. (3) A strong current of the suspension medium (arrows) stretches the blister which is now drop-shaped. (4) The blister is detached; no defect of the membrane is visible. (5) Formation of a new blister starts again.

It is interesting to note that the cellular part of the blister stalk becomes flattened out immediately after the blister is detached, and a new blister is formed at the same place where the original one was

located (Fig. 20). Under these circumstances, neither the formation of a hole in the cellular membrane nor the outflow of the protoplasm has been observed. The site of the origin of the detached blister becomes completely invisible. Occasionally, new blisters are formed in the base of an already existing blister. These newly formed daughter-blisters grow into the old blister (Fig. 21), and some of them even may develop in the wall of the mother-blisters, especially under the influence of certain chemicals (see below).

Experimental Alterations in the Process of Blister Formation

In order to determine the influence of the suspension medium on blister formation, suspensions of Brown-Pearce and V2 carcinoma cells were studied in normal rabbit serum and rabbit plasma as well as in BGR and isotonic salt solution. The same was done with frog cells of various kinds in frog serum, and the artificial mediums mentioned above. Blisters developed in all of these tests, although somewhat faster in the cells suspended in physiologic saline solution than in those in serum and plasma. There was no other difference in the process of blister formation in the various suspensions.

The cellular membrane disappears in cells suspended in 0.1 to 0.5 M ammonia. If, at the commencement of the experiment, blisters are present (Fig. 22), they are filled up by the swollen protoplasm and the nucleus as soon as 0.05 M ammonia reaches the cells, but the membrane remains visible (Fig. 23). Later, new blister formation may start in such a cell. The cellular membrane is not dissolved by 0.05 M ammonia, but swells, and sometimes daughter-blisters are formed within the membrane itself (Fig. 24). The formation of blisters is relatively independent of the pH of the medium (pH 2.3 to 10.0), but in a medium of pH 10.2 and higher, the membrane disappears and the contents of the blister flow out.

The cellular membrane very often becomes indistinctly outlined in dilute acetic acid, and the slightest pressure on the coverslip is sufficient to destroy the membrane. This change is irreversible. The ciliated epithelial cells show a particular form of reaction to acetic acid (Fig. 25): the cilia, instead of being straight and parallel, become irregular in form and arrangement. The basal bodies appear very dark, and occasionally the entire row of the basal bodies is separated from the rest of the protoplasm by a bright halo (Fig. 25). Therefore, the bases of the cilia behave like an independent section of the cellular membrane which does not shrink as much as does the rest of the membrane.

The formation of new blisters and their further growth is greatly

accelerated by distilled water (Figs. 19, 26, and 27). At the same moment that the distilled water reaches the cells under the coverslip, the nucleus and, to a lesser degree, the protoplasm swell so much that the whole blister is filled up in a few seconds. An instant later, the formation of new blisters starts. The black granules in such blisters swell considerably, particularly in detached blisters (Figs. 19 and 26). By this method it is possible to produce blisters even in cells in mitosis (Fig. 28), although they rarely develop in such cells in physiologic saline solution or in BGR.

Hypertonic salt solution, 0.9 per cent saline of pH 4.0, 70 and 95 per cent alcohol, and acetone, cause a very rapid shrinkage of the blisters, which goes on until the blisters have disappeared and the blister wall lies close to the protoplasm. In the course of this shrinkage, the blister wall becomes slightly wrinkled. In hypertonic salt solution, new blisters appear several seconds later; they grow very rapidly for several seconds and are morphologically indistinguishable from blisters in fresh cells. In the other media mentioned above, no further blister formation occurs, and even the replacement of the chemicals by physiologic saline solution does not produce a detachment of the cellular membrane.

The temperature of the suspension does not play a rôle in the process of blister formation: the amount and the size of the blisters are the same whether suspensions of the same mammalian cells are kept in the ice box, or at 37° or 41° C.

In cells which die spontaneously in the suspension, and in cells killed by heat or by chemicals, blister formation never occurs. Blisters, which are already present before the cells die, do not disappear, but they show no further growth. They remain visible until the cells disintegrate.

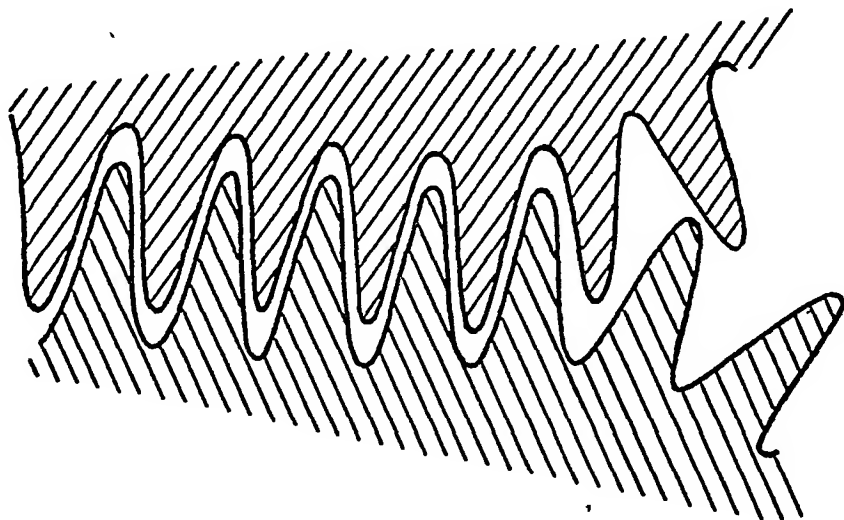
The Extrusion of Blisters from Renal Epithelium

Suspensions of kidney cells occasionally may contain some intact tubules held together by the basement membrane, in which case blisters develop only on the interior surface of the epithelium. The blisters grow very rapidly, and old blisters are very soon detached by the formation of new ones. Thus, free blisters are continuously expelled through the open ends of the tubules (Fig. 29). The same process was observed in unstained frozen sections of living kidney tissue, prepared from a human kidney removed surgically. The unfixed tissue was immediately put into physiologic saline solution and sectioned by means of a freezing microtome with a special knife-cooling device. The

sections were mounted in physiologic saline solution, and the edges of the coverslip sealed with vaseline. Slight, but distinct blister formation was visible in some of the tubules after a few seconds (Fig. 30).

The Cellular Membrane of Squamous Epithelium

Squamous cells were found to have a particular kind of cellular membrane. Suspensions of squamous cells were obtained by scraping the surface of the investigator's oral epithelium with the edge of a coverslip (Bosshard⁸ and von Albertini⁹). The membrane of these



Text-Figure 3. Schematic drawing of the interdigitating wrinkles of the surface of two squamous epithelial cells. On the right is shown the artificial detachment of the two cells.

cells was rather thick and very stiff, thus maintaining the irregular shape of the cells. The membrane was dissolved by 0.5 M ammonia after 1 or 2 hours, whereas the other chemicals mentioned above did not cause any alteration of the membrane. The surface of these cells is delicately wrinkled (von Albertini) and its pattern resembles a fingerprint (Fig. 32), whereas the contour is serrated (Fig. 31). The height of the wrinkles is about $0.5\ \mu$, their width 0.1 or $0.2\ \mu$. The distance between two wrinkles is approximately $0.3\ \mu$. As far as I could see, these wrinkles are folds of the superficial layer of the cellular membrane, interdigitating with those of the adjacent cells (Text-Fig. 3).

DISCUSSION

Meltzer,¹⁰ on theoretical grounds, introduced the expression "poto-cytosis" for the drinking or sipping of submicroscopic quantities of

water by cells. Later, the term "pinocytosis" was used to describe the intake of whole drops of fluid by "ruffle cellular pseudopodia" in tissue cultures (W. Lewis¹¹). The word "potocytosis" seems preferable for the process of blister formation described in the present paper since an intake of entire drops of fluid by pseudopodia was not observed.

The observations described in this paper demonstrate that potocytosis is a very common process in cells in suspensions. It occurs in epithelial as well as in mesenchymal cells and in normal cells as well as in tumor cells. Squamous cells were the only type which did not show potocytosis. Even distilled water, and 0.05 M ammonia, which increase blister formation greatly in other cells, did not bring about potocytosis in squamous cells.

A process very similar to blister formation was described by Hogue¹² in cells of tissue cultures that were exposed to hypertonic salt solution. However, sometimes blisters developed even in normal Locke-Lewis solution. The detachment of blisters also was observed in Hogue's experiments. Margaret Lewis¹³ described the formation of "blebs" along the edges of tissue cultures exposed to alkali. Similar "blebs" or sacs containing moving granules were observed by the same author in dying cells. These findings indicate that blister formation is not restricted to cells in suspensions.

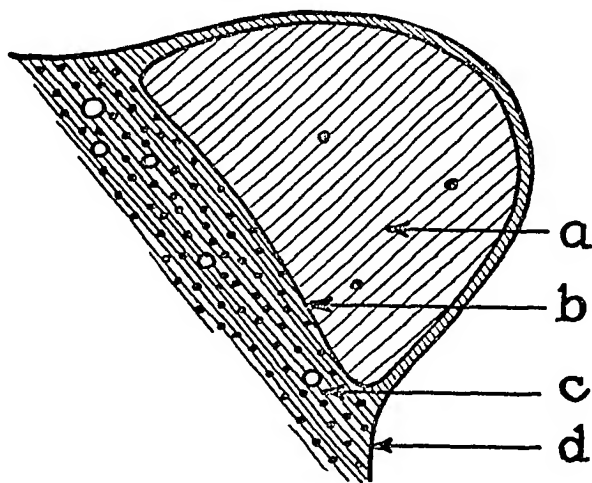
Since blisters fail to develop in cells which are completely surrounded by other cells in tissues, potocytosis must be considered a special type of fluid intake by living cells, which occurs particularly under unnatural conditions, *i.e.*, in suspensions and on the edges of tissue cultures. Therefore, it would appear that one of the fundamental conditions for potocytosis is the presence of a free cell surface in direct contact with an excess of fluid.

Furthermore, the formation and the enlargement of blisters are dependent on a second factor—the viability of the cells—for the enlargement and the new formation of blisters stop immediately when the cells die. This fact indicates that blister formation is an active function of the living cell and even a very low osmotic pressure of the medium is not sufficient to produce blisters in dead cells.

There is only a quantitative difference in the process of blister formation in cells in physiologic saline and those in hypertonic saline solution, distilled water, or homologous and heterologous serum. Hence the chemical constitution of the suspension medium seems not to be of fundamental importance for the process of potocytosis. The acceleration of blister formation in distilled water probably is a consequence of the greater osmotic pressure of the protoplasm as compared with

that of the medium. Under inverse circumstances, the cells being in molar NaCl, the blisters disappear due to the negative osmotic pressure. The subsequent rapid new formation and enlargement of blisters, which at first seem to contradict this explanation, are probably due to an increase in the permeability of the cellular membrane caused by a change in it. The process of blister formation was independent of the temperature of the suspension between 8° and 45° C.

The contents of the blisters seem to be part of the cellular protoplasm in sol-form, whereas the rest of the protoplasm consists of the different granules embedded in the ground substance, which here is a colloid in polyphasic form. Therefore, the granules originally located in the jelled cortex of the protoplasm (Chambers⁷), which is dissolved during the course of potocytosis (gel→sol), are the only ones moving freely in the blister contents later on. This fact proves that the distinct line of separation between the blister contents and the rest of the protoplasm (Fig. 16) is not a proper solid membrane, but a newly formed interface membrane. Therefore, the blister must be considered to be part of the protoplasm, and the blister contents to be outlined by an interface membrane (Text-Fig. 4). The free part of the blister wall behaves optically, as well as in its reaction to chemicals, like the



Text-Figure 4. Schematic drawing illustrating the structure of a cellular blister. (a) Blister contents with a few granules. (b) Internal interface membrane. (c) Protoplasm of the cell. (d) External interface membrane of the blister (plasma membrane).

cellular membrane in blister-free cells. It must be assumed that in regard to their physical and chemical structure these two membranes are identical.

The ability of living cells to form blisters is very interesting in regard to the problem of glandular secretion, for, although the conditions in the experiments reported above are unnatural, it is conceivable that

the secretion of at least some glands takes place in a manner very similar to potocytosis. A process resembling the formation and the detachment of blisters in suspensions has been observed by Jackson¹⁴ in cells of renal tubules of rats which had been fed a very high-protein diet. This author considered blister formation *in vivo* to be a sign of cell injury. The process is not restricted to kidney cells, since Jackson also saw it in uterine glands during pregnancy. Furthermore, the formation of blisters in renal tubules as observed in frozen sections of living tissue is similar to the process of "granuloid formation" in renal tubules (Kosugi¹⁵). Bell's assumption¹⁶ that the "granuloid" is an artifact of extracellular origin is disproved by the above-mentioned observations (see Fig. 30). Although we know the blisters to be a product of living cells, it is not possible at the present time to decide whether potocytosis in kidney cells is restricted to cells surviving the death of the individual or whether it occurs, either under physiologic or under pathologic conditions, while the individual as a whole is alive.

The phenomenon of potocytosis and the reaction of the cellular membrane to various chemicals aid in forming conclusions concerning the nature of the cellular membrane. NaOH (pH 10.2), and 0.1 to 0.5 M ammonia dissolve the cellular membrane, whereas in alcohol, acetone, and, to a lesser degree, in formalin, and in potassium bichromate, the cellular membrane shrinks, probably due to precipitation. Since the protoplasmic ground substance shows the same reactions to these chemicals, the chemical structure of the cellular membrane is likely to be identical or at least very similar to that of the protoplasm.

From the observations presented above, it appears that the membrane of the cells in internal organs is a thin film of a slightly sticky fluid rather than a solid membrane. Otherwise, it would be impossible to explain the fact that a defect caused by the detachment of blisters is immediately closed. A process similar to the detachment of blisters already has been described by Chambers¹⁷: Fat droplets may pass through the cellular membrane (plasma membrane) after intraprotoplasmic injection without causing a visible defect in the membrane. Danielli,¹⁸ using an oil-water interface model, was able to demonstrate the same phenomenon, thus assuming the cellular membrane to be merely an interface membrane. The cellular membrane does not behave like a semipermeable membrane either; the black and the brilliant granules are influenced and changed by hypertonic as well as by hypotonic salt solutions (Fig. 19), thus proving that the membrane is permeable to salts.

The phenomenon of "pinocytosis" (W. Lewis¹¹) is another proof that the cellular membrane cannot be solid. Therefore, the plasma membrane, which represents the only cover of the majority of the cells of the inner organs and of many tumors, has to be considered a simple interface membrane between the cytoplasm and the surrounding medium. The observation that blisters usually do not merge may be interpreted as a consequence of their surface tension. The same force prevents the blisters from rupturing.

The stiff membrane of the squamous cells seems to be identical with the "extraneous cellular integument," Chamber's "proper cellular membrane,"⁷ although I was not able to distinguish it optically from the hypothetic underlying plasma membrane. The observation that these cells fail to develop blisters suggests that the lack of an extraneous cellular integument is a third conditional factor for the formation of blisters.

SUMMARY AND CONCLUSIONS

Potocytosis, the process whereby visible blisters form on tissue cells suspended in liquid media, has been described in detail. Squamous cells alone amongst those studied did not exhibit blister formation, whereas this was almost invariably seen in normal and neoplastic cells of other types when one or more of their surfaces had remained in contact with an excess of fluid for a few minutes. The process of blister formation was accelerated in distilled water and in hypertonic solutions. The blister contents seemed to consist of highly diluted protoplasmic ground substance which was separated from the rest of the protoplasm by an interface membrane. When blisters were detached, the cellular membrane remained apparently intact. It is conceivable that some glandular cells pour out their secretions by the detachment of blisters, and that the "granuloids" seen in renal tubules by Kosugi¹⁵ and others may be detached blisters.

The findings as a whole seem to support the theory advanced by others that many of the cells of the internal organs and those of some tumors are outlined by an interface membrane that lies between the protoplasm and the surrounding medium. Furthermore, the permeability of the cellular membrane to hypotonic and hypertonic saline solutions, as indicated by visible changes in the cytoplasm of cells suspended therein, seems to be much greater than is commonly supposed. The integument of squamous cells, by contrast, seems to be a genuine stiff membrane which is folded into minute wrinkles that interdigitate with those of adjacent cells.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 100

FIGS. 1 to 4 show various preparations of human tumor material (adenocarcinoma of the stomach, biopsy) as seen with the ordinary and the phase microscope (PM). $\times 700$.

FIG. 1. Dry smear stained with Giemsa stain; ordinary microscope. The nuclear and protoplasmic elements are very indistinct and the cells are markedly shrunken.

FIG. 2. The edge of an unstained frozen section of fixed material; PM. The nuclear constituents are readily visible, whereas the protoplasm, due to the formalin effect, is granular.

FIG. 3. Paraffin section of Zenker-fixed material; hematoxylin and eosin. The cells have shrunk and the nuclei are irregularly outlined. The nuclear elements are less distinct than in Figure 2; the protoplasmic structure is about the same. (Ordinary microscope.)

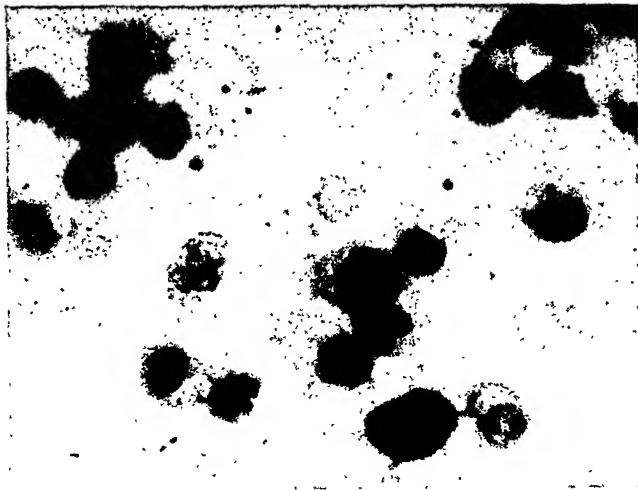
FIG. 4. Unstained cells in suspension; PM. The cells are not shrunken, the elements of the nuclei and the protoplasm being readily visible. *a* demonstrates a giant nucleus, *b* shows a multinucleated cell, and *c* illustrates a mitotic cell (metaphase).

FIG. 5. Fresh suspension of frog kidney cells. The elements are distinctly outlined by the cellular membranes. The protoplasm is filled with small, dark granules and the nuclei are "clear," containing a few dark dots. PM. $\times 700$.

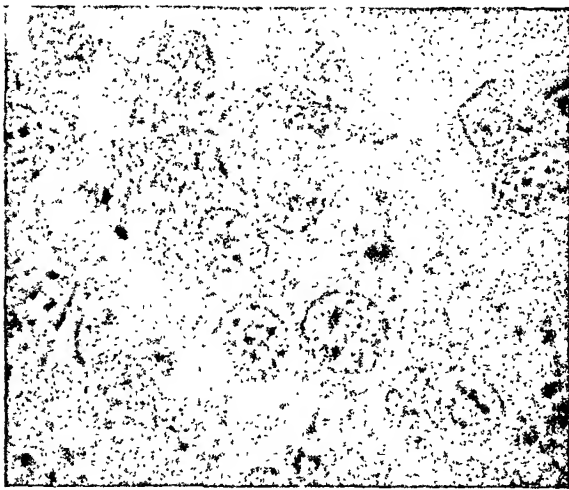
FIG. 6. Piece of a renal tubule of a frog. The cellular membranes are easily seen, while numerous small, black granules are visible in the cytoplasm of all of the cells. In the peripheral cells they are round and enlarged, while in the central area they are more rod-like. PM. $\times 700$.

FIG. 7. Cylindrical cell of the stomach, surgical specimen. There are three types of protoplasmic granules visible in the homogenous ground substance: (1) five large, white, spherical droplets on the left of the nucleus; (2) a group of very small, black granules in the apical pole (on the left) and near the base of the cell (on the right); and (3) many dull-gray granules in the basal part. PM. $\times 1400$.

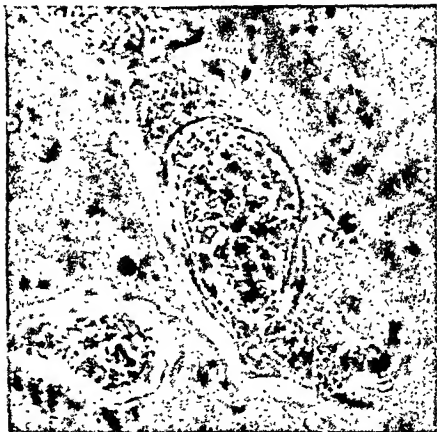
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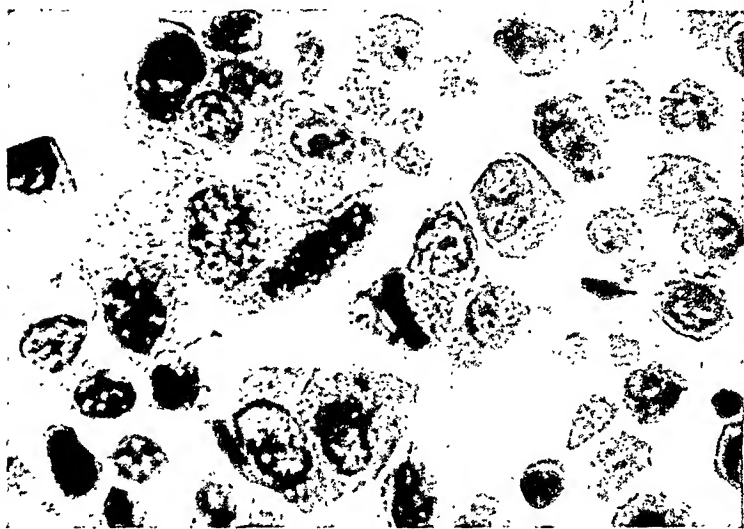
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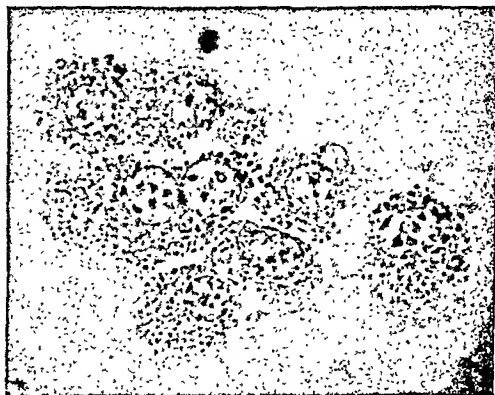
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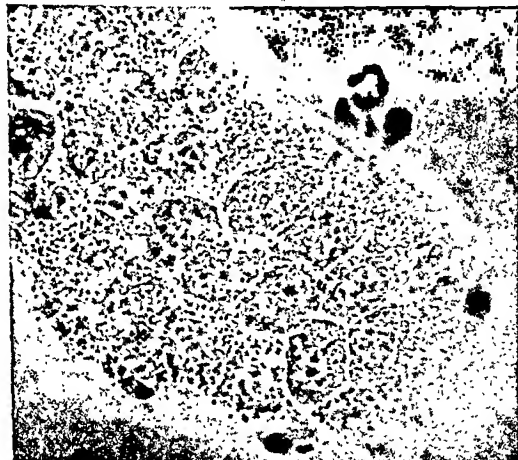
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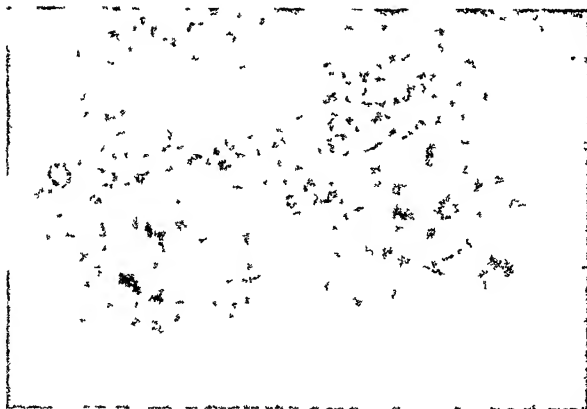
PLATE 101

- FIG. 8. Brown-Pearce carcinoma cells, after having been in the suspension under the coverslip for 30 minutes, showing extensive blister formation. In the right middle cell there are several small blisters in the contents of a large blister. Above this cell there is a free blister. The process of detachment of a blister can be seen in its terminal phase below the cell just mentioned. PM. $\times 700$.
- FIG. 9. Intraprotoplasmic origin of a blister (on the right) in a kidney cell of the frog, 4 minutes after the animal was killed and the suspension was made. PM. $\times 1400$.
- FIG. 10. Brown-Pearce carcinoma cell containing one intraplastic and one bulging blister. PM. $\times 1400$.
- FIG. 11. V2 carcinoma cell showing diffuse blister formation. The protoplasm is compressed by the blister contents; the latter appear slightly darker than the suspension medium. The blister is surrounded by a bright diffraction ring. PM. $\times 1400$.
- FIG. 12. The same cell as shown in Figure 11, 10 minutes later. The blister is markedly enlarged, and a second bright ring between the protoplasm and the blister contents has developed. PM. $\times 1400$.
- FIG. 13. Granulosa cell tumor showing marked diffuse blister formation after the suspension was in the ice box for 24 hours. The cell in the center, besides being "ballooned" diffusely, contains a spherical vacuole, the contents of which are much brighter than that of the blisters. PM. $\times 1400$.
- FIG. 14. Rabbit sarcoma cell exhibiting a single "local" blister. Of note is the distinct line between the blister contents and the rest of the protoplasm. PM. $\times 1400$.
- FIG. 15. Blister formation on the edge of a cortical piece of a frog kidney, 2 hours after the suspension was made. Some of the blisters are detached and stick on the surface of other blisters, but they do not merge. Several small granules are visible in some of the blisters. PM. $\times 850$.
- FIG. 16. Large blister in a kidney cell (frog) containing some relatively large, black granules (out of focus). The blister contents are separated from the compact protoplasm by a distinct line. PM. $\times 1400$.

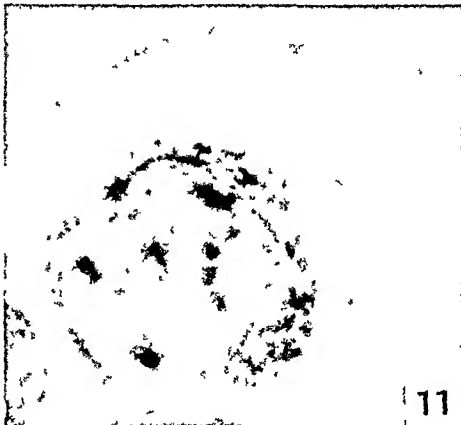
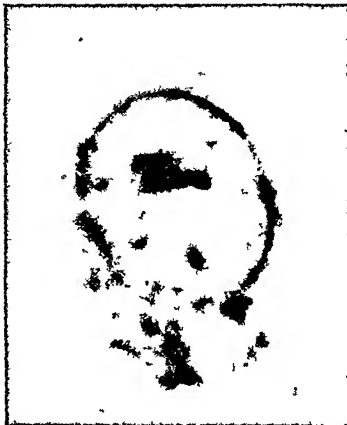
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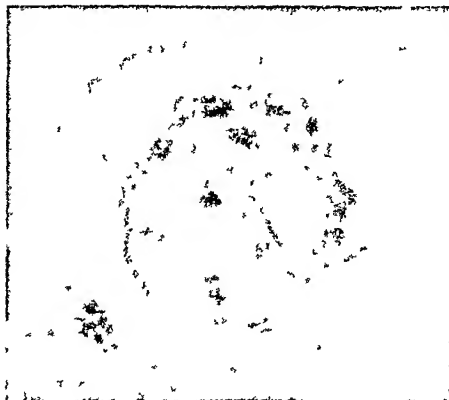
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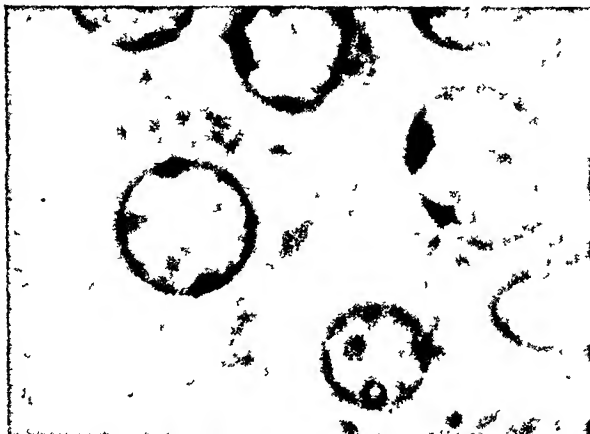


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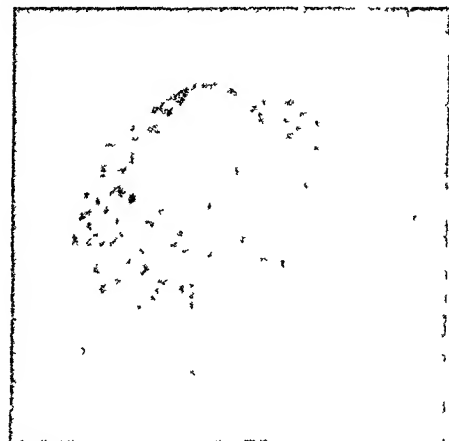


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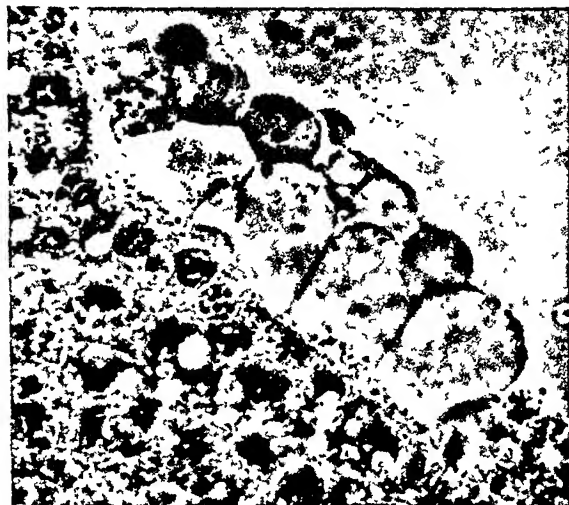
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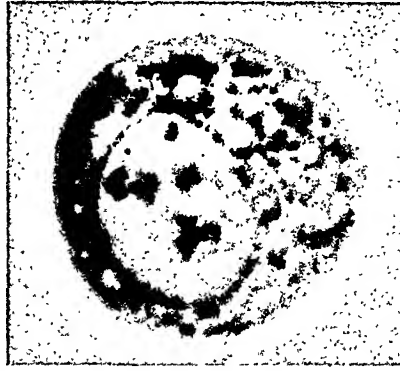
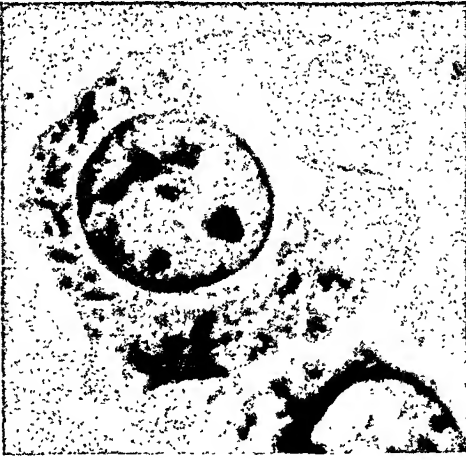
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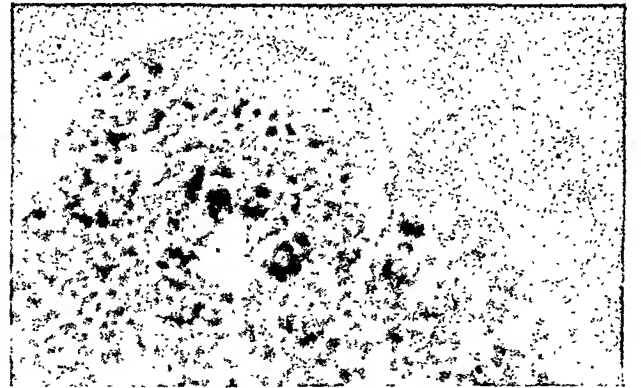
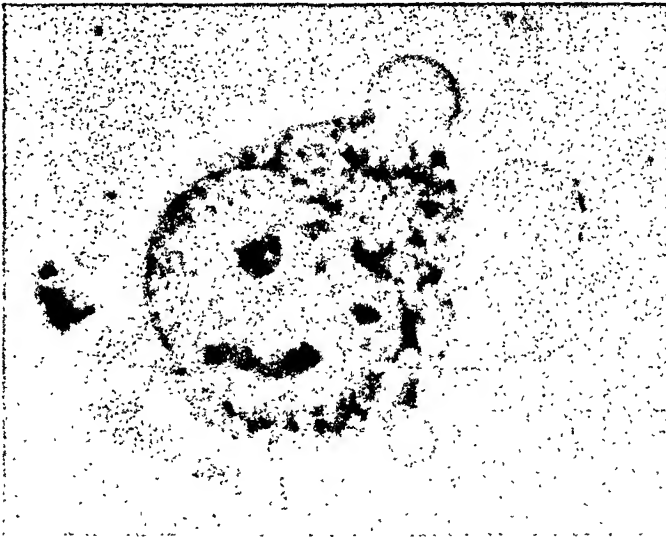
PLATE 102

- FIG. 17. Degenerated Brown-Pearce carcinoma cell after having been in the suspension at 37° C. for 5 hours. There are numerous small, bright vacuoles in the protoplasm. PM. $\times 1400$.
- FIG. 18. Brown-Pearce carcinoma cell. An artificial current of the suspension medium under the coverslip from the left to the right deflects two blisters, but they do not merge. PM. $\times 1400$.
- FIG. 19. Artificial blister of a ciliated frog cell in distilled water. On the left is a detached blister, containing some enlarged dark granules. PM. $\times 1400$.
- FIG. 20. Kidney cells of the frog. Formation of a new blister may be seen in the cell on the left after a blister has been detached previously. The new blister again contains some black granules. PM. $\times 1400$.
- FIG. 21. Brown-Pearce carcinoma cell showing numerous blisters, two of which have developed within the contents of a large blister. PM. $\times 1400$.
- FIG. 22. Ciliated cells of the frog pharynx. The chromatin network is out of focus; large spontaneous blisters have developed. PM. $\times 1400$.
- FIG. 23. The same cell as shown in Figure 22, 3 minutes after ammonia has been added to the suspension. The cilia have begun to disintegrate, the small, black, intraprotoplasmic granules (see Fig. 22) are very indistinct. The nucleus is enlarged and its membrane has almost disappeared. PM. $\times 1400$.
- FIG. 24. Frog kidney cell in 0.05 M ammonia, showing a large blister. The blister was already present before the ammonia was added to the suspension, but the ammonia caused a marked enlargement of the nucleus, which now fills the whole blister. A small new blister has been formed within the blister wall. PM. $\times 1400$.
- FIG. 25. The effect of 5 per cent acetic acid on a ciliated cell of the frog pharynx. The cilia are curled and their basal bodies are detached as a whole from the rest of the protoplasm. The nuclear membrane is double contoured and brilliant. PM. $\times 1400$.

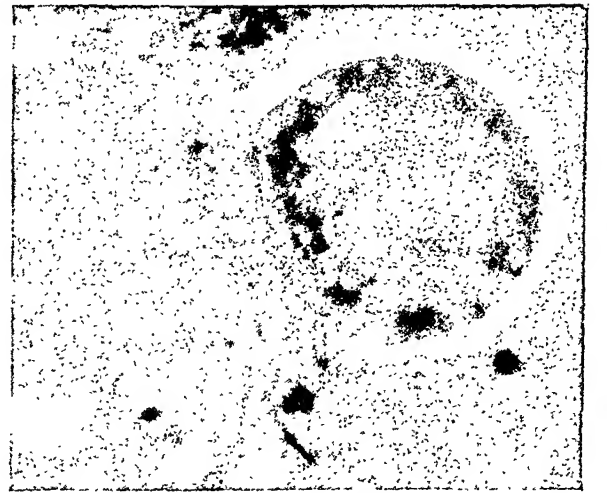
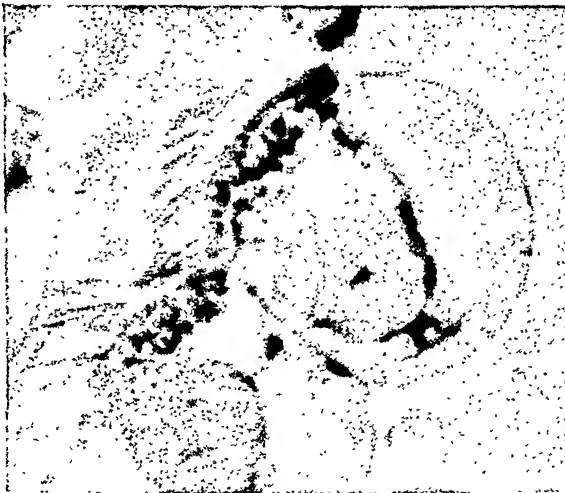
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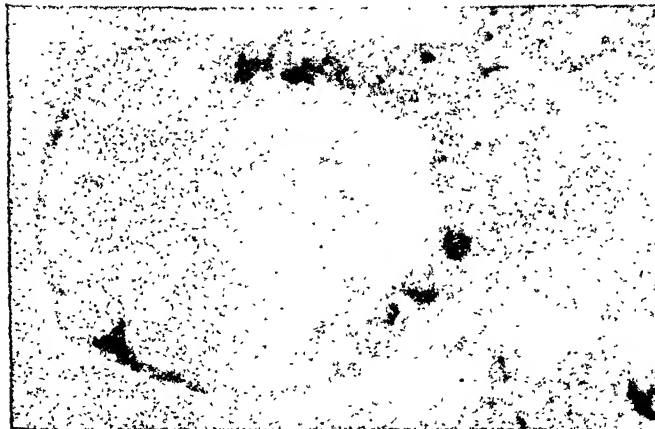


PLATE 103

FIG. 26. Brown-Pearce carcinoma cells with artificial blisters, caused by distilled water. The entire cellular membrane, stretched out by a high intracellular tension, seems to form the blister wall. The nucleus on the right is hazy and homogenous, its nucleolus as well as the nucleus of the cell on the left are out of focus. The protoplasmic granules are enlarged. PM. $\times 1400$.

FIG. 27. Artificial old blister of a C₃H sarcoma cell, which has been in distilled water for one hour. There are some small vacuoles in the compressed protoplasm. PM. $\times 1400$.

FIG. 28. Spontaneous blister formation in C₃H sarcoma cells. The cell on the right is in mitosis and its blister shows stalk formation. A bright halo surrounds the chromosomes, and small, black particles are present in the protoplasm of this cell. PM. $\times 1400$.

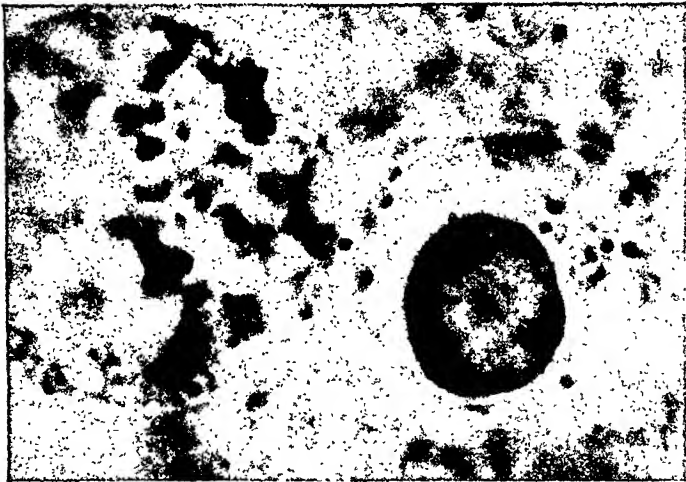
FIG. 29. An intact tubule of a mouse kidney has poured numerous blisters out of its open end. PM. $\times 700$.

FIG. 30. Unstained and unfixed frozen section of the cortex of a human kidney in physiologic saline solution. The section was made immediately after the surgical removal of the organ: the cells are still living and show blister formation into the lumen of a tubule. PM. $\times 400$.

FIG. 31. Cells of the same cell type as shown in Figure 32, in profile. The wrinkles are rather high and very sharp; the protoplasm contains many irregular, dark elements. PM. $\times 1400$.

FIG. 32. Upper surface of a squamous cell of the human mouth. The delicate wrinkles are clearly visible, as are the sharp edges of the cell. Some bacilli stick on the cellular surface. PM. $\times 1400$.

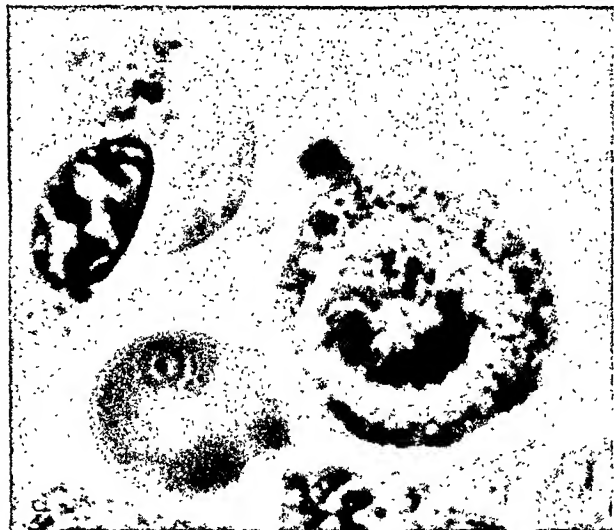
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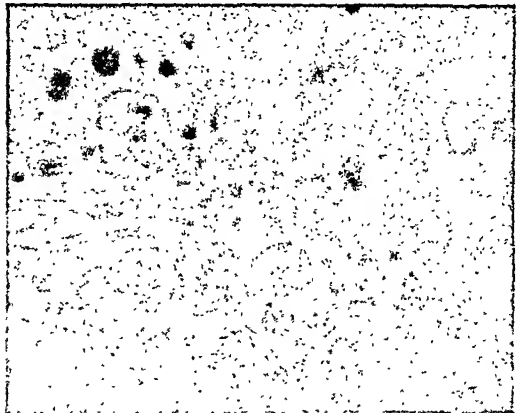
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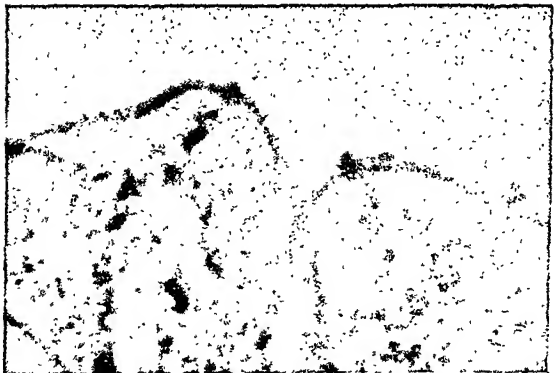
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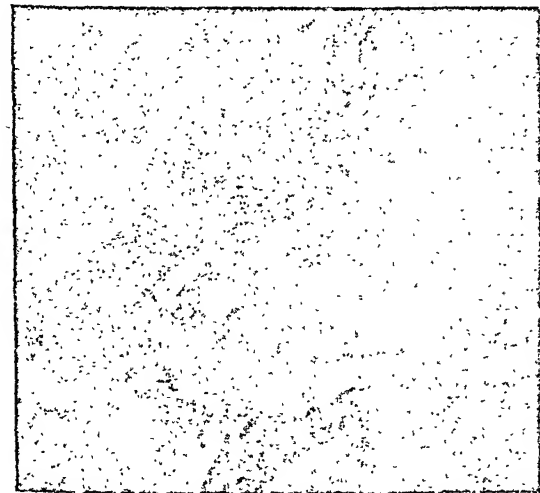
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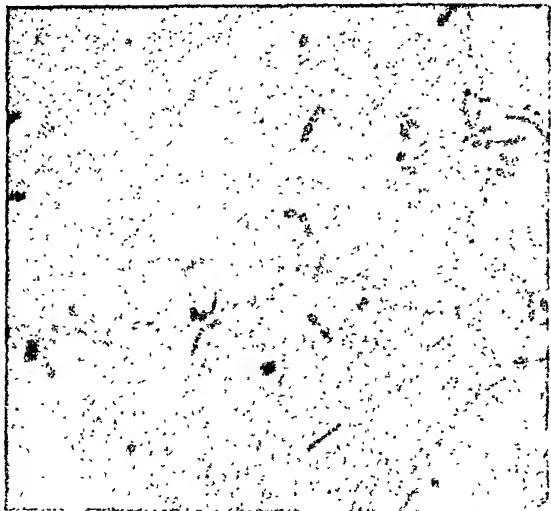
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3



Zollinger

Phase Microscopy, Potocytosis



CYTOLOGIC STUDIES WITH THE PHASE MICROSCOPE

II. THE MITOCHONDRIA AND OTHER CYTOPLASMIC CONSTITUENTS UNDER VARIOUS EXPERIMENTAL CONDITIONS *

HANS U. ZOLLINGER, M.D.†

(From the Department of Pathology of Cornell University Medical College and the New York Hospital, New York 21, N.Y.)

It has long been known that formed bodies of various sorts are present in the cytoplasm of tissue cells, those called mitochondria or chondriosomes having especially attracted the enduring interest of cytologists. The aims of the studies herein reported have been to identify the constituents of the protoplasm that can be seen with the phase microscope, and to learn more about their structure and chemical composition.

OBSERVATIONS

The working principle of the phase microscope (PM), the technic, and the material used in the experiments were described in the preceding paper.¹ By means of the PM three elements have been seen in the protoplasm of a variety of nucleated cells as follows: (1) a gray, mostly homogenous ground substance; (2) numerous small, dull-gray or black granules; (3) a few brilliant bluish granules of various sizes and shapes.

Ground Substance

Using the highest possible optical enlargement ($\times 1400$), very small, barely visible granules sometimes are observed in the homogenous ground substance, but the protoplasm of living cells never displays a network structure. In dead or dying cells, however, the protoplasm appears as a very dense network of irregular, thin fibrils, too small to be photographed, although readily recognizable in fixed and stained cells.

Mitochondria

The black or dull gray granules seen in the protoplasm of cells apparently are mitochondria. When viewed with an ordinary microscope these same granules appear pale yellow, and are stained with Janus green.

When very fresh cells of the kidney and the small intestine are viewed with the PM, these granules are rod-shaped and about $0.1\ \mu$ wide and from 0.8 to $1.2\ \mu$ long. In kidney cells these granules, or

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† Fellow of the Swiss Foundation for Biological-Medical Fellowships.

mitochondria as they will be called henceforth, very frequently show a small bulb on one end. In cylindric cells, such as those of the intestine, the mitochondria usually are located in the basal parts of the cells. If there are rod-like mitochondria, they are often oriented vertically to the basal membrane of the renal tubules or to that of the intestinal glands. In liver cells the mitochondria are arranged around the nucleus in a radial manner.

When cells have been in the suspension medium for a few minutes, the mitochondria usually become round, with a diameter of from 0.25 to 0.7 μ . The longer the cells remain in the medium the larger and more spherical become the mitochondria. Low temperature (below 37° C.) delays the change.

The size of the mitochondria varies not only from cell to cell of the same type, but even from one mitochondrion to another, this variation occurring in normal as well as in tumor cells. There is no difference in size or form between the mitochondria of the kidney cells of well fed frogs and those in cells of frogs which are starving. Rod-like mitochondria seem to be rare in tumor cells.

The mitochondria usually move slowly to and fro in the protoplasm. The looser the protoplasm, the faster is this movement, and the larger the amplitude. Since the mitochondria of destroyed cells, floating freely in the medium, show the same movement, it is probably due to the brownian movement of the surrounding molecules. These free mitochondria disintegrate only after several hours at 37° C. Intermediate stages between fat droplets (see below) and mitochondria cannot be seen.

Figures 3 to 5 demonstrate the cellular reaction to distilled water. The cells swell immediately and become spherical. The mitochondria enlarge greatly (Fig. 2) and sometimes appear as small vesicles. This enlargement is particularly striking in mitochondria of destroyed cells floating freely in the suspension medium. The thin walls of the vesicles look like membranes, each showing one distinct black, regular thickening (Fig. 7), which can be observed only in profile. Since the mitochondria are always rolling in the moving suspension medium under the coverslip, it can be observed that there is such a thickening in every vesicular mitochondrion. The material forming the thickening seems to be located on the internal side of the wall of the vesicle in every case, and projects into the interior of the vesicles. Therefore, the outline of these vesicular mitochondria is always smooth. These changes are reversible, and the mitochondria decrease in size when physiologic saline solution replaces the distilled water, and eventually reach their

original size (Fig. 8). The whole process can be repeated several times, the result always being the same.

Occasionally, blisters¹ contain mitochondria which swell immediately when reached by the distilled water under the coverslip, even before the swelling of the intraprotoplasmic mitochondria (Fig. 9).

In 0.05 to 0.1 M ammonia the mitochondria enlarge greatly (maximal diameter, $3.2\ \mu$), and take the shape of spherical balls, or even vesicles, with slightly irregular walls. The distinct regular thickening observed in distilled water does not appear. This enlargement in ammonia is especially striking in those mitochondria that float freely in the suspension medium (Fig. 11). After a minute or two in ammonia, some of these swollen mitochondria merge and form spherical, black balls, the diameter of which ranges from one-tenth to one-fifth of that of the swollen nucleus. The replacement of the ammonia by physiologic saline solution brings about a slow shrinkage of the single mitochondrion, whereas the coalesced mitochondria do not change their size (Fig. 12). In 0.5 M ammonia the whole cell is transformed into a jelly, the slightest pressure on the coverslip causing disintegration of the cell, after which the mitochondria float freely in the suspension medium as a compact mass.

Ten per cent formalin causes the whole cell to shrink slightly, and the mitochondria become very small but do not change their shape. The shrinkage of the protoplasm is much more striking in 100 per cent formalin; the cells become irregular, round, and yellow. These changes are irreversible. Seventy and 95 per cent alcohol, acetone, and, to a lesser degree, 3 per cent potassium bichromate have the same effect. In 5 per cent acetic acid there is no recognizable shrinkage of the protoplasm, but the mitochondria swell markedly; the replacement of this agent by physiologic saline solution does not yield any further change. Two surface active compounds investigated (0.1 per cent hexylresorcinol S.T. 37,* and zephiran†) cause a marked swelling of the protoplasm; in hexylresorcinol the mitochondria shrink irreversibly, whereas they remain unchanged in zephiran.

In order to determine the effect of various pH concentrations on living cells, the pH of 0.9 per cent NaCl was changed by means of NaOH and HCl, respectively, and the pH was measured with a glass electrode. In a medium of pH 5.8 to 4.0, the mitochondria enlarge slightly and reversibly (Fig. 10); the same is true at pH 8.3. At a lower H-ion concentration (10.1) the mitochondria swell more than at 8.3, and about 2 minutes later the whole cell begins to disintegrate. The en-

* Sharp & Dohme; † Winthrop Chemical Co., Inc.

largement of the mitochondria in alkaline media is reversible if the cells subsequently are washed in buffered physiologic saline solution of pH 7.0.

At 37° C. the mitochondria enlarge progressively until they reach their maximal size after about 2 hours. Afterwards they rapidly decrease in size. At 45° C. the maximal diameter is reached after 5 to 10 minutes, and at 56° C. after only 2 minutes. At 75° C. the mitochondria do not enlarge. Suspensions which have been in the ice box show very small mitochondria, but if the suspension is placed in the water bath at 37° C., the mitochondria enlarge much faster than do those of the fresh suspension.

Storage Granules

In fresh suspensions of normal organs the protoplasm very often contains several small, brilliant granules, which may be called "storage granules" (see discussion for explanation). These storage granules enlarge considerably when the suspension is kept at 37° C. for several hours. The enlargement is accelerated by high temperature (not exceeding 45° C.), and delayed by low temperature (Fig. 13). These granules are bluish under the PM and yellow-green under the ordinary microscope, and they show a very distinct, black contour (Figs. 1 and 2). Usually, the storage granules are spherical, but sometimes their shape is slightly irregular. Their diameter ranges from 0.1 to 2.0 μ . In liver and kidney cells, these brilliant granules are large and very numerous; in the stomach and the intestinal epithelium, their size and number are moderate, whereas in the ciliated epithelium and particularly in fibrocytes they are very small and infrequently found. In these latter cells they enlarge only slightly at 37° C. In tumor cells the brilliant granules are less numerous than in normal cells, but sometimes they are very large; usually the nuclei of such cells exhibit signs of degeneration. In most cells the storage granules are scattered throughout the protoplasm, but a particular arrangement of these granules around the intracellular mucus or, if there is no secretion, in the apical pole of the protoplasm is found in the cells of the stomach and the intestinal epithelium.

The storage granules sometimes move to and fro in the protoplasm, but not as much as do the mitochondria. The granules, after destruction of the cells, float freely in a physiologic medium and do not change their size or shape.

In suspensions of living cells, the storage granules are not stained by neutral red in a concentration of 1:10,000, but they stain deep red in cells which show disintegration of the nuclei or if too strong a con-

centration of the dye is used. In the latter case, the nuclei are damaged by the dye. If the cells are crushed by pressure on the coverslip, the storage granules remain almost unchanged (Fig. 14).

Distilled water, 0.05 M ammonia, 4 per cent formalin, 3 per cent potassium bichromate, and 0.1 per cent hexylresorcinol do not alter the storage granules. In 0.1 to 0.5 M ammonia, 5 per cent acetic acid, acidic media of pH 4.0 and higher, acetone, 95 per cent alcohol, and 0.1 per cent zephiran, the granules remain unchanged for some minutes. Then, suddenly, they disappear one after the other. However, there remains a wrinkled, thin, black shell for every granule. This can be demonstrated particularly well in granules floating freely in the medium. Unfortunately, these shell-like remains are too small (0.2 to 0.3 μ) to be shown in photomicrographs. With the exception of 0.1 M ammonia, this partial dissolution is irreversible in all of the experiments. In a solution of 0.1 M ammonia, the shell-like remains of the storage granules swell somewhat when the ammonia is replaced by physiologic saline solution, and they become spherical again, but never brilliant. Occasionally, neighboring storage granules may merge before being partly dissolved; in this intermediate phase they form large, brilliant, spherical globules. Molar saline solution causes a slight decrease in size of the storage granules combined with irregularities in shape; these changes are irreversible.

Granules of a particular kind can be seen in squamous cells of the epithelium of the mouth. Figure 15 demonstrates such a cell showing a pyknotic nucleus and numerous irregular, black granules. In other cells of the same origin the granules are much larger, brilliant yellow, and almost spherical, with some small indentations or sharp edges (Fig. 16). They never show brownian movement. In suspensions of Shope papilloma cells (Fig. 17) and, to a lesser degree, in those of the V2 carcinoma (originating in the Shope papilloma) these granules are very large and numerous. They show the typical staining reactions of keratohyalin (Ladewig and Oberndorfer²).

Microsomes

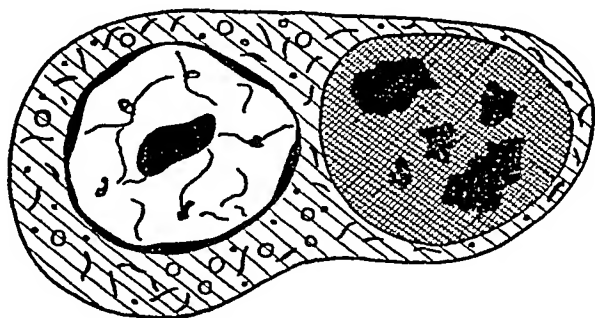
Blisters may contain some mitochondria and even storage granules.¹ In addition, under the influence of distilled water some very small particles appear in the blister contents. These particles move to and fro, and may almost reach the size of mitochondria in normal cells. The replacement of the distilled water by physiologic saline solution produces a sudden shrinkage and disappearance of the particles. These elements also can be observed in the blister contents when 0.05 M

ammonia has been applied, but they are more indistinct than in the experiments with distilled water.

After many failures, success was attained in photographing these minute particles. Apparently, when the particles are exactly in focus, they are too small for photographic reproduction, whereas, when the picture is focused on a plane slightly higher or lower than the particles, they appear as small, indistinct, black spots on the film. The results of making several exposures of the same cell and focusing at different levels are demonstrated in Figures 9 and 18.

Fat Droplets

Some cells, especially those of the liver and kidney, sometimes contain small or large (0.5 to $3.0\ \mu$), brilliant yellow droplets which are absolutely spherical and show a dark double contour (Fig. 19). When



Text-Figure 1. Brown-Pearce carcinoma cell containing a large "inclusion body," which shows large fragments.

the cells have been destroyed, these droplets float freely in the suspension medium. They never change their form and size during the experiments and do not move within the cells. Since the droplets are immediately dissolved by 95 per cent alcohol and selectively stained by scarlet red, there seems little doubt that they are composed of fat.

Inclusions

Cells of the rabbit sarcoma, the Brown-Pearce and V2 carcinomas, as well as those of the C₃H sarcoma, often contain large protoplasmic inclusion bodies. They are mostly spherical or oval and bright yellow (Fig. 20). When the suspension contains neutral red, 1:10,000, very often large, bright red granules appear within the inclusion bodies. Occasionally, these inclusion bodies contain several black, irregular particles which measure from 1 to 3 μ in diameter (Text-Fig. 1). It seems probable that these bodies represent dead cells, the black particles being fragments of their nuclei.

Vacuoles

Sometimes there are large, more or less rounded spaces in the protoplasm of cells which have been in suspension at 37° C. for several hours, or under the coverslip for 20 to 30 minutes (Figs. 21 and 22). They are irregularly outlined, and their contents stain slightly with neutral red. In unstained preparations the contents of vacuoles are much brighter than those of blisters.¹ These vacuoles never cause bulges of the cellular membrane, and do not contain particles. Therefore, the vacuoles are readily distinguishable from blisters.

In the ciliated epithelium of the frog pharynx and in a few tumor cells, round spaces occasionally may be seen located on the poles of the nuclei (Fig. 23). These spaces are less distinct, but even brighter than the vacuoles; it is possible that they represent the Golgi apparatus, but positive proof of this suggestion is not available. On the other hand, pictures like those presented by Brice, Jones, and Smyth,³ showing the Golgi apparatus as a large dark mass, were not observed in the experiments mentioned above.

DISCUSSION

The protoplasmic ground substance, in which the nucleus and the various granules are embedded, is said to consist mainly of a framework of fibrous proteins, as well as nucleoproteins with lipids and a mesh-filling interparticular fluid (Bensley,⁴ Guilliermond⁵). Claude⁶ assumed that the microsomes form the chromophilic part of the ground substance. Under normal conditions, this substance appears homogeneous and slightly gray under the PM; but it becomes net-like when the cells die as a result of heat. The sudden shrinkage and the fact that the protoplasm becomes irreversibly brilliant when placed in acetone, alcohol, etc., indicate that these chemicals cause a precipitation. It is very difficult to decide whether the ground substance is a sol or a gel. Lewis⁷ demonstrated the reversibility of the protoplasmic gelation produced by acid mediums and the dissolution of the protoplasm by alkali; however, in the experiments mentioned above these changes were irreversible. The brownian movement of the various protoplasmic particles, which may be seen in almost every cell, has led Bayliss⁸ to the conclusion that the protoplasm is a sol, whereas Lewis⁷ observed brownian movement only in cells whose protoplasm had been dissolved by alkali. As a matter of fact, the brownian movement differs very much from cell to cell, and even from particle to particle. This indicates that the protoplasm is a colloid in a polyphasic state, of which the proportion of the two phases (gel and sol) may change very easily.

The mitochondria can easily be observed in living cells by means of the PM; they are small, black, dull granules which may or may not show brownian movement. The shape of the mitochondria changes from cell to cell; rod-like mitochondria are rather rare in cells suspended in artificial mediums. The mitochondria react very rapidly to changes of the medium. Their enlargement in hypotonic solutions has already been observed by Anitschkow⁹ and by Bang and Sjövall.¹⁰ The vesicular swelling caused by the action of distilled water probably is a preponderantly osmotic effect; but no chemical constituents of the mitochondria can be observed to be dissolved by distilled water. In every freely floating vesicular mitochondrion there appears in distilled water a peculiar, strictly localized thickening of the wall (Fig. 7). Therefore, this thickening must be an integral constituent of every mitochondrion; it seems to represent the original body of the mitochondrion, the membrane or integument of which has become detached by the action of the distilled water. A comparison between this process and potocytosis¹ is obvious: a localized increase of the intracellular and especially the intraparticular water content takes place. Mirsky and Pollister¹¹ stated that molar NaCl dissolves desoxyribonucleoprotein selectively. The experiments reported above suggest that molar saline solution does not dissolve any constituent of the mitochondria. Provided that Mirsky and Pollister's assumption is correct, this indicates the lack of soluble desoxyribonucleoprotein in the mitochondria. This result, obtained by the direct optical method (PM), corresponds with the former findings of these authors,^{12,13} who used chemical analysis and an indirect optical method (microscopic sections), as well as with those of Caspersson and Santesson¹⁴ (microspectroscopy). Osmosis may partly explain the action of ammonia on the mitochondria, but their coalescence in ammonia indicates that the surface tension of the mitochondria decreases under the influence of ammonia.

The observation of a vesicular structure of the swollen mitochondria harmonizes with those of Guilliermond,⁵ Anitschkow,⁹ Hertwig,¹⁵ Cowdry,¹⁶ and Ludford.¹⁷ These morphologic findings seem to support the statement that the chemical constituents of the mitochondria, the lipid and protein molecules particularly, are concentrated in the superficial zone of each mitochondrion (Bensley,¹⁸ Bourne¹⁹). Observations with the electron microscope (Claude and Fullam²⁰) confirm the existence of a membrane forming the external zone of the mitochondrion. Opie and Lavin,²¹ using Giemsa's stain and ultraviolet light, have proved in recent investigations that the external layer of mitochondria consists of ribonucleic acid. Lewis⁷ and Hogue²² found

such vesicular mitochondria in tissue cultures treated with acids and anisotonic salt solutions, respectively. Both authors considered cells containing vesicular mitochondria to be dead.

Since the shrinkage of the mitochondria in alcohol and acetone is irreversible, it may be assumed that alcohol and acetone dissolve the lipid constituents, and perhaps precipitate the proteins.

The reaction of the mitochondria to heat (45° to 65° C.) indicates that these temperatures first accelerate and then delay a process, which takes place in every cell of a suspension at 37° C., and even at room temperature. The higher the temperature, the sooner it occurs. The visible sign of this process is the enlargement of the mitochondria. The significance of this enlargement is unknown; it might be caused by the change of the intracellular H-ion concentration as a result of pathologic cellular metabolism in an unnatural surrounding.

Chambers²³ succeeded in breaking mitochondria into two pieces with the microneedle; Strangeways and Canti²⁴ and Claude⁶ observed mitochondria spontaneously breaking in two. Therefore, the contents of the mitochondria are not likely to be in a fluid state.

When reviewed in their entirety, these experiments and reflections lead to the following conclusion: the mitochondrion consists of an elastic membrane and its contents which, under ordinary conditions, are probably in gel form. Important components of the mitochondria seem to be located in, or just below, the membrane. When the mitochondrion swells by intake of water (in distilled water, etc.), the membrane is detached from the "body" of the mitochondrion by the fluid.

Finally, the fact deserves emphasis that the mitochondria react rather rapidly to every chemical change of the medium, but are not very fragile; very often they "survive" the mechanical destruction of cells for some time.

The "storage granules" are spherical or slightly irregular, brilliant granules with a bluish color in the PM. They are very resistant to mechanical influences, and thus the suspension medium usually contains a great number of these particles. The number of storage granules is surprisingly high in liver and kidney cells (storage granules: mitochondria = 2:1, or 1:1). The discrepancy between this statement and the findings in microscopic sections, in which von Möllendorff²⁵ described extremely rare granules in kidney cells, can be explained easily by the dissolution of large parts of the storage granules by fixatives and dehydrating chemicals (see below). The differential diagnosis between storage granules and fat droplets as observed with the PM can be made easily by means of alcohol: fat is immediately and

completely dissolved, whereas the storage granules disappear later, leaving shell-like remains.

The reaction of the storage granules to various chemicals (alcohol, acetone, acetic acid, etc.) indicates that these granules consist of at least two different materials. One component, which gives the storage granules their bluish color and their brilliancy in the PM, and the yellow color in the ordinary microscope, is dissolved under the influence of these chemicals. The other component is probably precipitated by these agents, and remains visible in the form of a "shell." Claude,⁶ using differential centrifugation, found that phospholipid and ribonucleic acid were the main constituents of the storage granules of liver cells. Therefore, the storage granules must be considered to consist of a solid membrane and its fluid contents, which are rich in lipids. The solid structure of the membrane is proved by the facts that (1) the storage granules may be irregularly outlined, and (2) a wrinkled "shell" remains after the action of the chemicals mentioned above. The contents of the storage granules must be fluid, or at least not solid, because the granules burst immediately when punctured by a micro-needle (Chambers²³).

Due to the small size of the "shells," the determination of their chemical composition is difficult. The main material is probably protein. Deane,²⁶ in a recent paper on the basophilic bodies in hepatic cells, demonstrated that these bodies consist of ribonucleoprotein. These bodies may be identical to the remains or "shells" of the storage granules. If this is true, the variability of these bodies in size and form (Deane) could be a consequence of the different sizes which the storage granules had reached at the moment of fixation, as well as of the degree of their disintegration caused by fixation and dehydration. In further experiments I shall try to decide whether my assumption is correct.

The storage granules are by no means a homogenous group of granules; without doubt this group includes elements of different functional properties:

(1) Proof of the secretory function of storage granules of one type is offered by Claude,⁶ who observed an accumulation of the "secretory granules" in liver cells of the starving *Amphiuma tridactylum*. After the animal's first meal, these granules were poured out. A cyclic change of the "secretory granules" in gland cells is assumed by Maximow and Bloom,²⁷ and many others. The general conception is that these genuine secretory granules store the secretion products, or their precursors, until they are poured out.

(2) It was stated above that the storage granules observed in these experiments enlarge in living cells in suspension, and that this enlargement depends upon the temperature of the suspension medium as well as upon the length of time which the cells stay in the suspension. In fresh suspensions, on the other hand, particularly in those of malignant tumor cells which have been thoroughly washed in physiologic saline solution and then stored in a protein-free medium, the main building material of the storage granules has to be considered of endocellular origin. It has to be assumed, therefore, that granules of this particular type store accumulated waste products of the cellular metabolism. The same statement was made years ago by Lewis and McCoy,²⁸ who proposed to call these particles "starvation granules," whereas Ludford¹⁷ named them "degeneration granules." *In vitro*, a secretion of such granules never was observed, and their further fate *in vivo* is unknown also. It is conceivable that such cells, as, for instance, in the neighborhood of an infarct, can recover and later remove the granules.

(3) A further subgroup is formed by the "resorption granules," which have been studied particularly by Oliver²⁹ in the cells of renal tubules. He demonstrated the accumulation of large protein granules in cells of certain sections of the nephron after intraperitoneal injection of heterologous protein into animals. These granules in all probability represent heterologous protein, resorbed by the tubular cells from the primary urine after having passed through the glomeruli. These "resorption granules" are a common finding in human glomerulonephrosis (Zollinger³⁰). The fundamental process leading to the formation of resorption granules is the cellular resorption and the intracellular accumulation of a primarily extracellular material.

It has been emphasized that the storage granules are not a homogeneous group; however, the experiments reported above demonstrate that the optical behavior as well as the structure of the three subgroups in the PM are the same. Furthermore, the three subgroups are founded on the intracellular storage of either exogenous or endogenous material, probably containing proteins. For these reasons, the concentration of the three subgroups in one main group, called "storage granules," seems to be justified.

According to these theories, the granules of squamous cells would represent storage granules, which consist in this case of keratohyalin. These brilliant particles appear first in the deepest layers of the multilayered squamous epithelium, where the mitochondria are well preserved. In the upper layers they are enlarged and the mitochondria

disintegrate. Therefore, a close connection between the mitochondria and these keratohyaline granules is unlikely to exist (Cowdry³¹).

It has not yet been decided whether there is a connection between the mitochondria and the storage granules in general. Von Möllendorff,²⁵ Maximow and Bloom,²⁷ and many others considered them to be fundamentally different, whereas Oliver²⁹ and Bloom³² defended the theory of a transformation of mitochondria into storage granules, at least as far as the above-mentioned resorption granules in cells of renal tubules are concerned. Through the courtesy of Dr. Jean Oliver, I had the opportunity to study such cells and tubules with the PM. It is certainly true that the number of mitochondria is remarkably decreased in cells containing large resorption granules. But the evidence that the resorption granules are formed from fully developed mitochondria is not convincing. In the experiments with various cell suspensions it was observed that the storage granules can be very small in cells exhibiting relatively large mitochondria. The storage granules, therefore, must originate in particles which are much smaller than normal mitochondria.

An intermediate position between the two extreme opinions is taken by Claude,⁶ who assumed that the mitochondria and the secretory granules "constitute extreme forms in a continuous series of cytoplasmic elements." When dealing with the significance of the microsomes, this opinion shall be mentioned again.

A further observation to be discussed is the appearance of very small granules in the blisters of cells which are suspended in distilled water or in 0.05 M ammonia. Two similar observations are reported by Hogue²² and Chambers and Fell.³³ These particles presumably are identical to microsomes (Bayliss⁸) or to ultramicroscopic particles (Bensley⁴). Of course, Hertwig's original definition of microsomes as particles, the size of which is (under ordinary circumstances*) below the resolving power of a light microscope,¹⁵ leaves one undecided whether or not it includes several kinds of particles. As a matter of fact, two submicroscopic elements of different chemical composition have been recognized in liver cells (Lazarow³⁴). Since the microsomes measure from 60 to 150 μ (Claude⁶), it is obvious that they are scarcely visible under the ordinary microscope, as well as under the PM; but the slightest enlargement brings them within the range of visibility. This fact is utilized fully by the PM, which allows them to be observed without any shrinkage caused by fixation. Further-

* Words in parenthesis were added by the author.

more, the PM makes it possible to select blisters containing separated microsomes for observation.

A similarity between the microsomes and the secretory granules, as suggested by Claude,³⁵ could not be noticed in my experiments. The optical picture of microsomes, as well as their behavior in different mediums, is contrary to those of secretory granules. In recent studies, Claude⁶ observed the breakdown of mitochondria into small "microsome-like elements," and Baker³⁶ pointed out that microsomes are chemically very similar to the mitochondria.

Future investigations may help to decide whether there is a fundamental difference between mitochondria and microsomes, or whether microsomes are an intermediate stage in the formation of mitochondria *de novo*. The above-mentioned opinion of Claude⁶ with a slight modification, seems in best agreement with the sum of the numerous known facts. Accordingly, microsomes would have to be regarded as the original, undifferentiated particles, which, corresponding to the needs of the cell, may develop into mitochondria as well as into secretory granules.

The bright intraprotoplasmatic vacuoles which occur exclusively in cells of old suspensions and of disintegrating tumors have nothing in common with the blisters.¹ They are probably the result of protoplasmic autodigestion, as suggested by Cowdry.¹⁶

There seems to be little doubt that the large inclusions in tumors represent necrotic tumor cells in which the remains of the disintegrated nuclei are sometimes still visible as dark fragments. The different stages of this necrophagocytosis can be observed in a suspension of a highly necrotic V2 carcinoma. The dead cell body is enveloped by the protoplasm of a living tumor cell. The large granules, stained with neutral red, are probably storage granules which developed before the cell had died.³⁷

SUMMARY

A study of the protoplasmic constituents of various cells with the phase microscope has led to the following observations and conclusions:

The mitochondria appear as small gray or black, indistinctly outlined, spherical or rod-like granules. Normally, they appear as solid particles, but in distilled water and in dilute solutions of ammonia they enlarge greatly, and fluid accumulates between a superficial interface membrane and the slightly swollen "body" of the mitochondrion. In normal cells some of the mitochondria exhibit brownian movement.

Mitochondria react readily when the cell is exposed to adverse environmental conditions. They are not fragile, however, and often may be seen for some time after the mechanical destruction of the cell.

Storage granules are usually spherical or slightly irregular and have a brilliant blue hue. Their fluid contents are surrounded by a thin membrane, which may be seen as a "shell" after the destruction of the granules. They may enlarge in a number of ways: by the accumulation of waste products (degeneration granules), by preparing cellular products for secretion (secretory granules), or by resorption (resorption granules). The zymogen granules, the hyaline droplets in renal tubular cells, and the irregular keratohyaline granules of squamous cells belong to this group of cytoplasmic constituents.

The microsomes cannot be seen in the cellular cytoplasm except in the blisters produced by distilled water or ammonia. These visible microsomes are probably greatly swollen. It is conceivable that microsomes may be undifferentiated precursors of mitochondria and storage granules.

The protoplasm of cells which have been in suspension for a long time contains irregular degeneration vacuoles. They are bright and have nothing in common with blisters.

No Golgi apparatus could be identified with the phase microscope in these experiments.

Several types of experimental tumor cells often contained large protoplasmic inclusions which seemed to represent phagocytized disintegrating cells.

It appears that the protoplasm is a colloid in a polyphasic state of sol-gel balance, and that this state may change very easily.

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DESCRIPTION OF PLATES

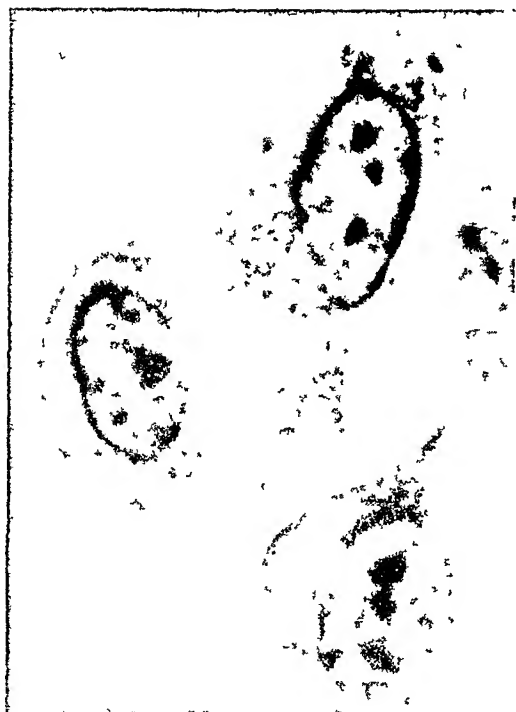
PLATE 104

- FIG. 1. Isolated kidney cell of the frog. The large, bright elements with black contour are storage granules; the indistinct gray granules are mitochondria. On the left there is a single fat droplet. The cellular membrane is not in focus. PM. $\times 1400$.
- FIG. 2. Brown-Pearce carcinoma cells in distilled water. The nuclei are hazy and moderately enlarged, and the mitochondria are swollen. The nucleoli of the two cells in the middle are out of focus. PM. $\times 1400$.
- FIG. 3. A group of unchanged Brown-Pearce carcinoma cells for comparison with Figures 4 and 5. PM. $\times 1400$.
- FIG. 4. The same group of carcinoma cells shown in Figure 3, 3 minutes after the replacement of the physiologic saline solution by distilled water. The nuclei are swollen and hazy, the nucleoli are indistinct (out of focus), and blister formation is accelerated. Enlarged, black mitochondria may be seen. PM. $\times 1400$.
- FIG. 5. The same group as shown in Figures 3 and 4, 4 minutes after further replacement of the distilled water by physiologic saline solution. The nuclei are smaller than in Figure 4; the chromatin network shows the same arrangement as in Figure 3, and the nucleoli have reappeared. The mitochondria have regained their original size. PM. $\times 1400$.
- FIG. 6. C₃H sarcoma of the mouse. The mitochondria and the storage granules are easily distinguishable from each other. The cell on the left contains two rod-like mitochondria near the lower pole of the nucleus. PM. $\times 1400$.
- FIG. 7. The effect of distilled water on mitochondria, which float freely in the suspension medium after mechanical destruction of frog kidney cells. The mitochondria are markedly enlarged and vesicular; the black, distinct thickening of the wall (see text) is readily visible in many of the vesicles. PM. $\times 1400$.
- FIG. 8. C₃H sarcoma cell after replacement of the distilled water by physiologic saline solution. The nucleus has lost its hazy structure and the chromatin network has reappeared. The mitochondria are still round, but not markedly enlarged. PM. $\times 1400$.

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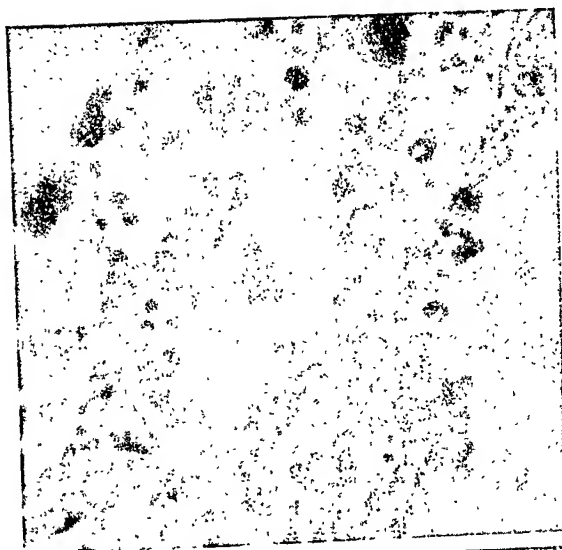
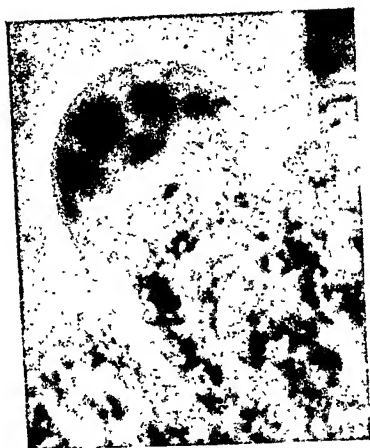
PLATE 105

- FIG. 9. Kidney cells of the frog in physiologic saline solution, to which distilled water had been added just before the picture was taken. The mitochondria in the protoplasm are still rod-like and small, whereas they are greatly enlarged in the blister contents. Besides the enlarged mitochondria, the blister contains two very small particles (microsomes). PM. $\times 1400$.
- FIG. 10. Brown-Pearce carcinoma cell in saline solution of pH 4.0. The nuclear membrane has become brilliant; the chromatin network is very dense; the nucleolus is enlarged; and the protoplasm has shrunk. PM. $\times 1400$.
- FIG. 11. Large, vesicle-like mitochondria and storage granules of frog kidney cells in 0.5 M ammonia. The cellular membrane has been destroyed completely, and the granules are markedly swollen. PM. $\times 1400$.
- FIG. 12. Ciliated epithelial cells of the frog, treated with 0.1 M ammonia, and washed afterwards in physiologic saline solution. Most of the mitochondria again are small, but where they had merged before, they did not decrease in size (large black dots). PM. $\times 1400$.
- FIG. 13. Brown-Pearce carcinoma cells, after having been at 6° C. for 3 hours. The mitochondria and the storage granules are very small; the cells show marked potocytosis. PM. $\times 1400$.
- FIG. 14. V2 carcinoma cells killed by pressure on the coverslip. The mitochondria have disappeared; the storage granules are well preserved, but very small. The nuclear membrane is no longer visible. PM. $\times 1400$.
- FIG. 15. Numerous irregular, black granules in an epithelial cell of the human mouth. The nucleus is slightly shrunken. Of note is the thick, irregular cellular membrane. PM. $\times 1400$.
- FIG. 16. A cell of the same type as shown in Figure 15. The granules are larger, shiny, and partly spherical. PM. $\times 1400$.
- FIG. 17. A cell from a Shope papilloma of the rabbit, containing granules of the same type as shown in Figure 16. The nucleus is out of focus. PM. $\times 1400$.

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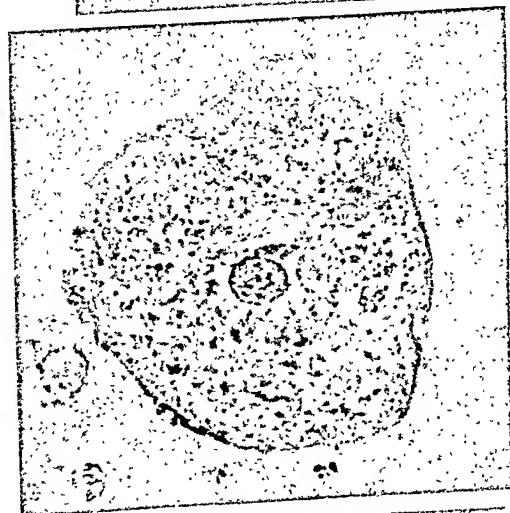


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Phase Microscopy, Mitochondria

PLATE 106

FIG. 18. Blister in a kidney cell of the frog, in distilled water containing two tiny, black granules (microsomes). PM. $\times 1400$.

FIG. 19. Liver cell of the frog filled with fat droplets and a few storage granules. PM. $\times 1400$.

FIG. 20. Brown-Pearce carcinoma cell with a large inclusion body. The bright ring around the body indicates that the latter is quite spherical. PM. $\times 1400$.

FIG. 21. Brown-Pearce carcinoma cells after having been in suspension at 37° C. for 3 hours. Blister formation can be seen in every cell. In addition, the cell on the left contains several vacuoles. The brilliant storage granules and the black or gray mitochondria are easily distinguishable from each other. The nucleoli are large, and the chromatin network is plump. PM. $\times 1400$.

FIG. 22. Brown-Pearce carcinoma cells of a fresh suspension. At the upper left there is a necrotic cell with a bright diffraction ring; next to it there is a degenerated cell with enlarged storage granules. The cell below shows vacuoles. PM. $\times 1400$.

FIG. 23. Rabbit sarcoma cell with two paranuclear "vacuoles" and a large blister. PM. $\times 1400$.

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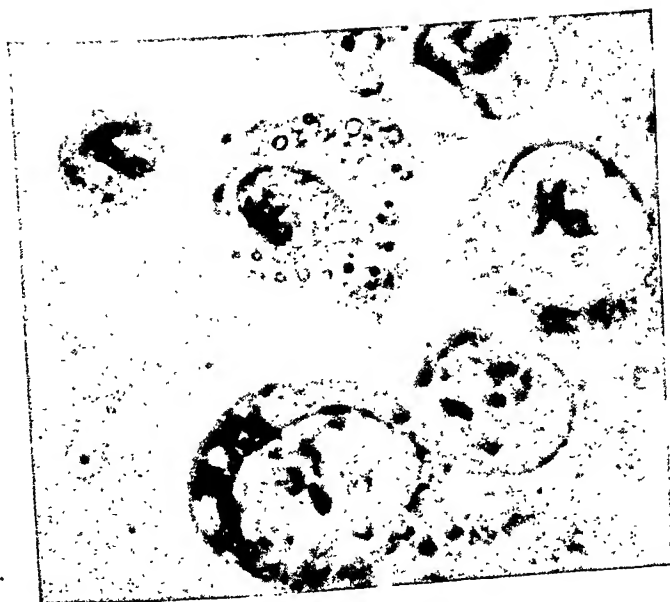
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Zollinger

Phase Microscopy, Mitochondria

MELANOMAS OF CHILDHOOD *

SOPHIE SPITZ, M.D.

(From the Pathology Laboratories of the Memorial Hospital, New York, N.Y.)

It has become apparent over a period of years that even when a histologic diagnosis of malignant melanoma has been made in children the clinical behavior rarely has been that of a malignant tumor. The disparity in behavior of the melanomas of adults and children, despite the histologic similarity of the lesions occurring in the different age groups, is obviously a matter of fundamental importance and the following questions immediately arise: Does the histologically malignant melanoma of children differ in any structural detail from that of adults? Can the clinical behavior of these lesions be predicted from their histologic structure? What, if any, are the factors known to influence the clinical behavior? Should the melanomas of children be treated any differently from the melanomas of adults?

MATERIAL

In a search of the files of the Memorial Hospital for instances of malignant melanoma in children, it soon became apparent that the diagnosis had been made with far greater frequency 20 or more years ago than in the past decade. This difference was quickly accounted for in the usual structure of the benign pigmented nevi of children as contrasted with that of the benign nevi of adults. In more recent years, the criteria for the diagnosis of malignant melanoma had become clarified to the extent that histologic features of the nevus of childhood, formerly regarded as stigmata of malignant change, were no longer so considered. However, there remained a group of cases in which a diagnosis of malignant melanoma seemed histologically sound. Over a period of years, the qualification has been added to reports of such lesions that they probably would not behave as malignant tumors. In order to distinguish these lesions both from the malignant melanoma of adults and the unequivocally benign nevus of childhood, the term "juvenile melanoma" has been adopted. The term "melanoma" in this paper, as in common usage, has been applied only as an abbreviation for malignant melanoma.

The material for this study is comprised of 13 cases † diagnosed histologically as juvenile melanoma during the past 13 years and occurring in children ranging in age from 18 months to 12 years. For

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† Submitted from the Mixed Tumor Service of the Memorial Hospital.

purposes of comparison, a group of melanomas occurring in young adults of from 14 to 19 years of age also was reviewed. In addition, 50 consecutive cases of benign nevus occurring in children ranging in age from 1 month to 12 years were included in the comparative study. Blue nevi (Jadassohn) and Mongolian spots were not included in this study since they form a recognizable entity usually easily segregated from malignant melanomas both in histologic appearance and in their generally benign clinical behavior.

Hematoxylin and eosin preparations of all lesions were available for study; in some instances silver stains and Masson's trichrome preparation also were used.

CLINICAL FEATURES

In the group of childhood melanomas (juvenile melanomas) there were 5 males and 8 females. Three were less than 2 years of age; one was 3 years of age; one, 5 years old; and the remaining 8 patients ranged in age from 8 to 12 years. The clinical appearance was varied: 10 of the 13 patients had lesions under 1 cm. in diameter and only 3 lesions were between 1 and 3 cm. In a few, the lesions were described as being smooth with sharply delimited edges, but in the majority they were verrucous with irregular margins (Figs. 1 to 4). All were elevated above the skin surface. The color was described as pink to red in 5 whereas 7 varied from brown to black. One lesion was said to have been subcutaneous. None was described as hairy.

The lesions had been noted for the duration of life in 6 cases but were said to have existed for from 6 weeks to 4 years in 7 cases. Three of the patients were presented for treatment within 1 year of the first appearance of the lesion. There was a history of gradual increase in size in all cases except one in which there was rapid growth for 6 weeks only.

Five of the lesions occurred on the face, one on the trunk, 2 on the upper extremity, and 5 on the lower extremity; only one of the latter category occurred on the sole. The parents of all these children stated that the lesions were in locations where they were frequently traumatized during the course of daily activities but none gave a history of frequent bleeding and none of the lesions was grossly ulcerated at the time of examination. Treatment consisted only of local surgical excision in all cases; in one case a group of obviously metastatic nodes was later removed from the groin.

All but one of the 13 children are alive and have shown no evidence of recurrence either locally or in drainage sites. They have been fol-

lowed clinically for periods up to 13 years. Only 2, both female, have been followed for as short a time as 3 years and both of these have now passed their menarche. The remaining 10 have been seen regularly for from 5 to 13 years; 6 of these have passed the age of puberty.

One of the 13 cases had been clinically malignant and the child is dead. This one fatality occurred in a female child whose lesion was first noted at the age of 12 years; there had been no development of secondary sex characteristics and she had not menstruated. This lesion occurred on the sole of the foot but was not described as involving the skin. After rapid growth over a period of 6 weeks, a soft white tumor, 2 cm. in diameter, was resected from the plantar fascia. One month after the initial excision there was a bulky local recurrence, thrombosis of the femoral vein, and metastasis to inguinal lymph nodes. Within 4 months the child was dead of generalized metastases.

HISTOLOGIC FEATURES

The epidermis was present in the sections studied in 12 of the 13 cases and was in all instances altered in a characteristic manner. Frequently there was hyperkeratosis and occasionally patchy parakeratosis. The epidermis immediately over the bulk of the tumor often was acanthotic and showed spongiosis, sometimes to so marked a degree that small intra-epidermal vesicles were present. There was superficial ulceration of the epidermis of 2 of the lesions; neither of these was malignant clinically. The rete pegs in 7 of the lesions were irregularly elongated and extended rather deep into the dermal tumor.

The most distinctive feature of the epidermal change was found in the basal layer, which was not uniformly palisaded as in the normal skin. The continuity of the basal layer was interrupted by scattered cells or groups of cells which were irregularly enlarged and distended by uniform fine brown granules. Similar isolated cells also were occasionally scattered irregularly in the acanthotic malpighian layer. These enlarged pigmented cells often were increased twice or more in size over normal basal cells; the nuclei were of varied size but were mainly large and vesicular. There was a loss of cohesion between these altered cells and the adjacent cells of the epidermis. This change, often referred to as the junctional or dermo-epidermal change, occurred diffusely over the entire surface of the tumor, but often there was added to the diffuse change a more distinctive alteration in which islands of large pigmented cells formed bulbous knobs and pegs which extended down into the dermis (Figs. 5 and 6). In places, the extensions from the epidermis seemed to be bounded by an intact basement membrane,

but in all lesions it was possible to trace direct continuity between them and the cells forming the dermal portion of the tumor.

Nine of the 13 cases presented a histologic appearance which in most respects was indistinguishable from the adult type of malignant melanoma. In 3 of these the lesions were relatively superficial and had infiltrated only to the level of the mid-dermis; in 6 there was infiltration through the entire dermis. The structure varied not only in the different lesions but also in any one lesion. The large cells distended with fine pigment, described in the epidermis, formed long projections into the dermis. These cells at times assumed a definite spindle shape in the infiltrating portion of the tumor. In several there were compact clusters of spindle cells, but this structure was predominant in only one case of this series, that is, the one fatal case. In the remainder, spindle cells were interspersed among large acidophilic cells which more often formed the bulk of the tumor. These cells were varied in size but were always large, rounded or polygonal, with vesicular nuclei and large acidophilic nucleoli (Fig. 5). There was either alveolar or perivascular arrangement of the cells.

In one feature alone some of these lesions were distinctly different from the malignant melanoma of adults. In 8 of the 9 cases just described, giant cells were present both in the epidermal and dermal portion of the tumor (Figs. 7 and 8). In 5 cases there were small to moderate numbers of these cells, but in 3 cases giant cells were present in such large numbers as to constitute the most outstanding feature of the lesion. These giant cells were totally unlike those formed by fused nuclei seen so commonly in the benign nevus. They were most prominent in the basal layer of the epidermis or in the superficial part of the dermal tumor and were either multinuclear or mononuclear. In the multinucleate cells the number of nuclei varied from four to six; generally they were in peripheral arrangement but occasionally were clumped in the center of the cells. The cytoplasm was acidophilic and sometimes granular. Pigment was seldom seen in the giant cells but commonly they contained vacuoles suggesting fat. Most of the giant cells were round or oval but often there were stellate cytoplasmic processes, particularly in those connected with the epidermis (Fig. 8). Silver stains have failed to show argyrophilic processes on these or other cells of the tumor; nor do trichrome stains indicate that they have origin in muscle.

In 3 cases, the dermal portion of the tumor was composed entirely of spindle cells, different principally from those described above in the large cytoplasmic content of the cells and the rather orderly inter-

lacing bundles of cells (Fig. 9). This structure bore strong resemblance to epidermoid carcinoma, particularly of the spindle cell type. The junctional change so constant a feature of the entire series also was present in this part of the group.

One case was especially different from the other 12. A 10-year-old boy had a black lesion on the lip, present since birth. He has been followed for 7 years and there has been no recurrence. This lesion had essentially the structure of a simple benign intradermal nevus with clusters and strands of cells extending into the subcutaneous fat. The distinctive feature was the almost uniform enlargement of each cell to a diameter three or four times that of an ordinary nevus cell. The increase in size was primarily in the amount of cytoplasm, which was peppered with fine melanin granules. The nuclei also were enlarged and hyperchromatic, but irregularly so. There were acidophilic nuclear inclusions which far outnumbered those in other lesions of this series. At the periphery of only three other lesions of this series were there cords and nests of small round tumor cells.

Mitotic figures were not prominent in any of these lesions but occasionally could be identified without any great difficulty both in the epidermal and dermal portions of the tumors.

Pigment was present in all of the lesions but only 3 were heavily pigmented. Melanin was far more prominent in the enlarged cells at the dermo-epidermal junction than in the other portions of the tumor but was present also in scattered tumor cells of the dermis, in the malpighian layer of the epidermis, in the parakeratotic scales and vesicles, and in the dermal chromatophores (Fig. 6). The differences in color noted clinically could not be correlated with differences in pigment content. Actually 2 of the most pigmented lesions were clinically red. The color variations were most easily accounted for on the basis of the vascularity of the tumor; that is, those that were red showed greater vascularity rather than less pigment.

The cutaneous appendages often remained intact in the tumor. The basal layer of the hair follicles participated in the junctional change which occurred in the epidermis, but to a lesser degree. The sebaceous and sweat glands were not altered except by distortion due probably to pressure of the surrounding tumor.

Associated with juvenile melanomas were inflammatory changes consisting in a few cases simply of a sparse infiltrate of lymphocytes and plasma cells at the periphery of the lesion. In other lesions the infiltrate was more intense and involved the tumor itself as well as the tissues surrounding the tumor. In 2 cases in which vesiculation and

ulceration of the epidermis were noted there were also polymorphonuclear neutrophils and eosinophils in the infiltrate.

In most of the tumors there was diffuse edema involving not only the epidermis but also the dermis, particularly the papillary layer. In places there seemed to be an almost complete dissolution of the basal layer and the tumor cells appeared to be floating in the edema fluid of the dermis. The capillaries of the papillary layer were dilated and engorged. The lymphatics of the dermis were also dilated, particularly at the dermo-epidermal junction.

Differentiation of Juvenile Melanoma from Benign Nevus of Childhood

The histologic sections of 50 unselected benign nevi of children were studied for purposes of comparison with juvenile melanoma. These nevi were removed chiefly for cosmetic reasons from children ranging from 1 month to 12 years of age, occurred in the skin in almost all regions of the body, and ranged from very small macules to large lesions that covered almost the entire trunk. All of these children are alive and none has shown recurrence over periods up to 7 years.

The ratio of incidence of juvenile melanoma and of the ordinary benign nevi of childhood is difficult to determine inasmuch as usually only the nevi of unusual clinical appearance are removed. However, an approximation of the relative incidence may be gathered from the fact that over a period of about 6 years 100 pigmented nevi of children were removed surgically; of these there were 8 juvenile melanomas, or a ratio of 1:12.

In contrast to the pleomorphic structure encountered in the group of juvenile melanomas, the benign nevi of childhood were monotonously alike, in most instances, in their histologic structure. The epidermis covering the nevus was generally thin but showed segments of acanthosis. There was increased pigmentation in all layers but the pigment was most concentrated in the basal layer. In 49 of the 50 lesions (98 per cent) there were scattered, somewhat enlarged, pigmented cells in the basal layer singly as well as in nests (Fig. 10) which extended into the dermis. The projecting nests were sometimes still bounded by a compressed rim of basal cells. One of the 50 lesions showed no alteration of the epidermis overlying the nevus.

As a rule, the benign nevus of children was far more cellular than the ordinary nevus of adults. The upper segments of the nevus were crowded with closely packed pigmented nevus cells which were of uniform size and shape; in the lower segments of the tumor there was

gradual diminution in the amount of pigment and in the size and number of cells, as well as increase in fibrous tissue. The deeper segments of the benign nevus in children were often composed of delicate strands of small nonpigmented cells surrounded by large collagenous bands. It also was noted that the structures resembling Meissner's corpuscles (lames foliacées), so commonly found in adult nevi, were practically absent in this series.

There are, then, definite cellular features of distinction between the juvenile melanoma and the ordinary benign nevus of children: (1) The pleomorphic structure of the juvenile melanoma is in contrast to the monotonous structure of the benign nevus of children; (2) In juvenile melanoma there are bizarre mononuclear or multinuclear giant cells totally unlike those formed by fused nuclei in the benign nevus; (3) The junctional change so prominent in the benign nevus is comprised of cells which are uniform, small, and closely packed whereas in the juvenile melanoma these cells are pleomorphic, larger, and form looser projections in the dermis; (4) Mitotic figures, occasionally seen in the juvenile melanoma, are rare in the ordinary nevus.

While the details of the problems of the morphogenesis of nevi are beyond the scope of this paper, certain features of differentiation between benign nevi of children and the corresponding lesion of adults are worth noting. There appears to be a remarkable difference in the incidence of junctional change in the nevi of the two age groups. This alteration was present in 98 per cent of the children included in this study and is in contrast to the reports of Allen¹ of 12 per cent and of Montgomery and Kernohan² of 25 per cent in their studies of adults. The pronounced cellularity in the nevi of childhood has led to the erroneous diagnosis of malignant melanoma just as the cellularity of hemangiomas of infancy has led to the diagnosis of angiosarcoma by those not familiar with the natural evolution of these lesions.

Differentiation of Juvenile Melanomas from Adult Melanomas

In view of the radical contrast in behavior between juvenile and adult melanomas, it seemed of interest to determine the life history and possible histologic variations of melanomas occurring in an intermediate age group. Accordingly, a series of 17 melanomas occurring in patients ranging in age from 14 to 19 years was used for comparative study. In this group there were 5 males and 12 females. Three of the lesions occurred on the face or neck; 5 on the trunk; 2 on the upper extremity, and 7 on the lower extremity (none on the sole). There was a history in all that the lesions had been growing for from 1 to 2 years

before local excision; some of these lesions had been present for a lifetime. All of the female patients had undergone menarche from 3 months to 4 years before the removal of the tumor. In several of the females there was a definite history that the pigmented cutaneous lesion had increased two to three times in size since the onset of menstruation which had occurred only 3 to 4 months prior to the removal of the tumor.

In the group of 13 "juvenile melanomas," only one patient is dead (7.7 per cent) whereas in a similar group of melanomas of 17 young adults, 12 are dead (71 per cent), the fatalities having occurred within 6 to 18 months after the initial diagnosis. An analysis of the 5 living patients reveals one with metastasis that has survived for 4 years. Four (23.5 per cent) have survived for periods of 5, 9, 11, and 17 years, respectively. The average 5-year survival for adults of all ages, as determined recently in a series of 595 cases,³ is 9.7 per cent. There is at least a suggestion in these figures, obviously in need of confirmation by a larger series, that perhaps melanomas occurring even in an intermediate age group carry a more favorable prognosis than those occurring at a later age.

In the fatal cases, ranging from 14 to 19 years, the variations in structure were so great that it was not considered possible to correlate prognosis with histologic appearance. However, several features of this group bear noting. In only one of the adults (Fig. 11) were there giant cells of the type that were identified in approximately one-half the group of juvenile melanomas. This patient has now survived for 5 years. Similar cells have been noted occasionally in adult nevi (not included in this study).

Although there was some tendency toward less pigmentation in the juvenile melanomas, this feature was too inconstant to be of diagnostic or prognostic significance. Mitotic figures were more numerous in the melanomas of the intermediate age group but they were present sufficiently often in the juvenile melanomas to make this latter lesion a definite exception to the rule that mitotic figures in nevi are evidence of malignant melanoma.

A generally appreciated feature that was again demonstrated was the lack of correlation between the depth of the local cutaneous infiltration of the lesions and the ultimate outcome. The lesions in several of the fatal cases of young adults were extremely superficial and some had a qualitative histologic appearance far less malignant than many of the nonfatal juvenile melanomas.

In general, it was concluded that differentiation histologically between the juvenile and adult melanomas could not be made with certainty in most cases. The one feature, found in almost one-half the cases of juvenile melanoma, that seemed to permit a histologic distinction from adult melanoma, was the presence of giant cells (Figs. 7 and 8). In view of the survival of patients having this type of tumor, these have been regarded as an indication that the lesion is benign. This is so despite the fact that, except for the giant cells, such lesions have all the histologic criteria for the diagnosis of malignant melanoma.

INCIDENCE OF MELANOMAS IN CHILDHOOD

Contrary to the general impression of the frequency of occurrence of malignant melanoma in children, a review of the recent medical literature reveals very few reports substantiated either histologically or by fatal outcome. Wells⁴ stated that "Although pigmented moles are frequently present at birth, they rarely become malignant before birth or even in infancy." He accepted only the case of Coe⁵ as a true congenital melanoma; this lesion occurred on the scalp of a newborn infant, grew rapidly, metastasized to nodes, and caused death in 4 months. Milian, Périn, and Brunel⁶ reported an instance of melanoma occurring in the parietotemporal region of the male, 12 years of age, but neither photomicrographic evidence nor follow-up data are presented as corroboration of the diagnosis. The case of Périn and Blaire,⁷ occurring on the cheek of a child, 3 years of age, appears histologically to have been melanoma but the child died of bronchopneumonia following whooping cough 7 months after the initial excision of the lesion so that clinical evidence of its malignant course is lacking.

Sweet and Connerty⁸ have reported a bulky tumor replacing the genitalia in an infant that also had a bathing trunk nevus; this child died shortly after birth and had hepatic and pontine metastases. The pontine lesion was heavily pigmented and the authors felt that the logical diagnosis was probably malignant melanoma. The recent report of Russo⁹ in which osseous metastases are described in 2 children, 5 weeks and 3 years of age, is not substantiated by photographic proof of the diagnosis, and the possibility comes to mind that these 2 cases might represent neuroblastomas rather than melanomas. The lesion in his third case, occurring in a Negro female, 5 years old, might well be a melanoma but this child has been well for 3 years after the excision of the tumor.

Webster, Stevenson, and Stout,¹⁰ however, mentioned 10 cases of

histologic melanoma which occurred in children under the age of 10 years. Only 2 cases are detailed specifically in their paper but neither is recorded as having been fatal. The outcome of the other cases is not stated but the authors do mention that lesions in children giving the histologic appearance of malignant melanoma rarely metastasize.

Callender, Wilder, and Ash,¹¹ in a review of 1600 ocular melanomas, recorded only 2 instances in patients from 0 to 9 years and 13 from 10 to 19 years. Although their follow-up data are admittedly incomplete, the youngest patient to die in their series was 19 years of age.

In the current study, the histologic diagnosis of juvenile melanoma has been made in 13 cases while only one of these has been clinically malignant. This one fatal case, occurring in a 12-year-old girl, was distinctly different histologically as well as clinically from the group as a whole. The tumor was composed entirely of nonpigmented spindle cells and involved primarily the plantar fascia (Fig. 12). Unfortunately, a section of overlying skin was not submitted with the primary tumor, but the metastatic lesions in inguinal lymph nodes were of the pleomorphic structure generally encountered in melanomas.

A case which was both clinically and histologically malignant recently was submitted to this laboratory by Dr. Bjarne Pearson of the Department of Pathology of the University of Vermont. This lesion occurred in a 9-year-old female child who was normally developed and showed no precocious sexual features. The pigmented lesion on the knee was only 4 mm. in diameter at the time of removal and, while it had been present for several years, growth had occurred over a period of only a few weeks. The cells of the primary lesion in this instance contained large, irregular, hyperchromatic nuclei with prominent vacuoles and acidophilic inclusions which would justify the diagnosis of malignant melanoma, regardless of the age (Fig. 13). Bilaterally, the inguinal nodes showed a few clusters of metastatic cells in the peripheral sinuses. Although the follow-up in this child has been only for a period of 6 months and it is not possible to predict the outcome, it has been noted that generalized dissemination of the tumor has become evident in the fatal cases of young adults and in the one fatal case among the children within a very short time after the diagnosis has been made. It seems possible, however, that in some cases metastases to regional nodes in children need not always indicate a fatal termination. This peculiarity of melanomas in children would seem to be indicated by the case included in the report of Webster, Stevenson, and Stout¹⁰; this child, 8 years old, after local excision of

a black lesion on the shoulder and subsequent metastases both to skin and cervical lymph nodes which were resected, had survived at least 12 years without further recurrence.

FACTORS INFLUENCING CLINICAL BEHAVIOR OF JUVENILE MELANOMAS

Inasmuch as there is a lack of constant morphologic evidence with which to explain the usually benign clinical behavior of histologically malignant melanoma occurring in childhood, an explanation based on sex-linked hormonal control would seem logically feasible. The peak of incidence of malignant melanoma occurs between the age of 40 and 60 years. Despite the fact that both cutaneous and ocular melanomas are relatively uncommon in younger age groups, there is too sharp a rise in mortality once the age of puberty is passed to be attributable to a general increase in incidence of cancer with age.

There is, moreover, in our experience, frequent recurrence of the clinical information that the growth of pre-existing nevi is greatly accelerated at the time of, or shortly after, puberty. At times these cases will follow a rapidly fatal course out of all proportion to the morphologic appearance of the lesion (Fig. 14). Two cases of malignant melanoma^{5,8} have been reported in the newborn in which ante-natal metastases have occurred; a variety of hormonal influences exist during this period which do not ordinarily obtain thereafter. There is some evidence¹⁰ that even though metastasis may occur in childhood, an inhibitory factor may exist before puberty to hinder either further dissemination or reception of metastatic cells by the viscera. Presumptive though this evidence may be, an intensive investigation of the possible influence of sex-linked hormonal alterations on the activation of melanoma seems mandatory.

SUMMARY AND CONCLUSIONS

Of 13 cases of juvenile melanoma in this series, only one (7.7 per cent) has had a clinically malignant and fatal course despite the similarity of the juvenile lesions to the malignant melanoma of adults.

The juvenile melanoma may be distinguished histologically from adult melanoma in about one-half the cases by the presence of giant cells in the former which seldom occur in the latter.

There is a precipitous rise in the capacity of melanomas to metastasize after puberty despite the histologic similarity to the usually non-metastasizing juvenile melanoma.

The possible influence of sex-linked hormonal activation of the growth capacity of melanomas at the age of puberty seems a logical conclusion.

Accordingly, since metastases from juvenile melanomas occur only rarely, conservative surgery, rather than the radical surgery usually indicated for adult melanomas, seems justified.

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DESCRIPTION OF PLATES

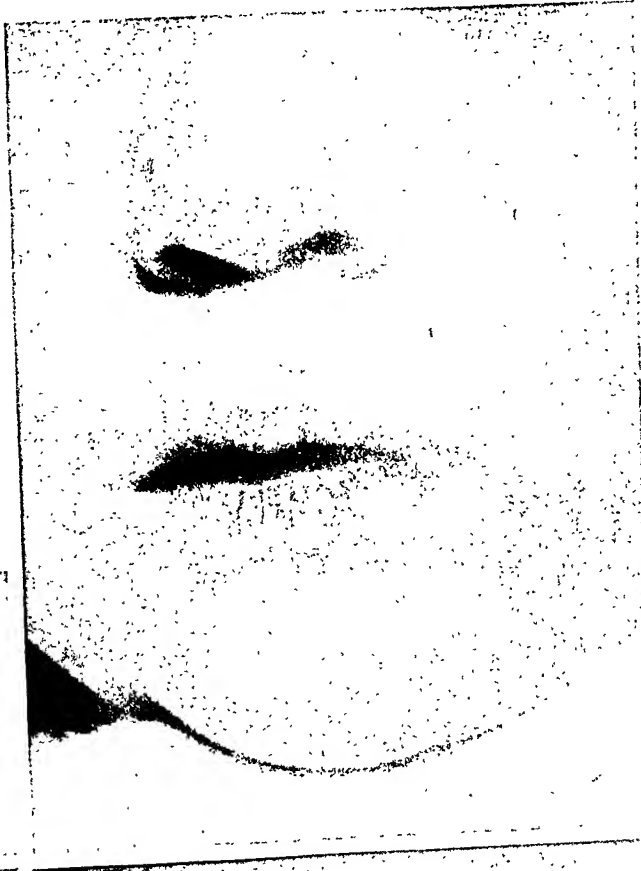
PLATE 107

- FIG. 1. Male, 20 months of age. Smooth red lesion on cheek present since birth.
- FIG. 2. Female, 20 months old. Smooth black lesion on lip noted for 2 months.
- FIG. 3. Male, 5 years old. Verrucous brown lesion on chest present for 6 weeks.
- FIG. 4. Female, 4 years of age. Rough black lesion on cheek noted for 1 year.

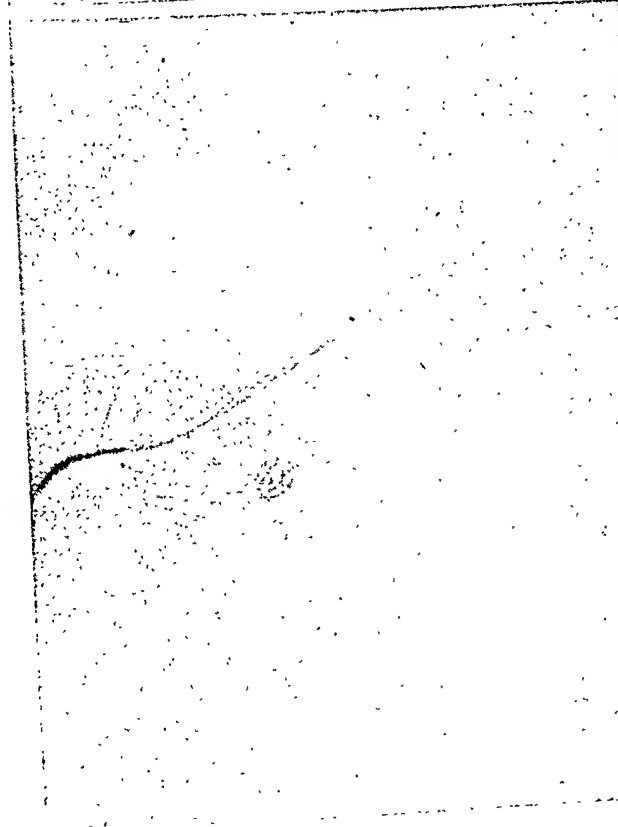
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Melanomas of Childhood

Spitz

PLATE 108

FIG. 5. Junctional alteration overlying juvenile melanoma formed by large pleomorphic acidophilic cells. Hematoxylin and eosin stain. $\times 220$.

FIG. 6. Heavily pigmented tumor on the thigh of an 11-year-old female, showing junctional alteration and pleomorphic infiltrating tumor. Hematoxylin and eosin stain. $\times 180$.

5



6



Melanomas of Childhood

PLATE 109

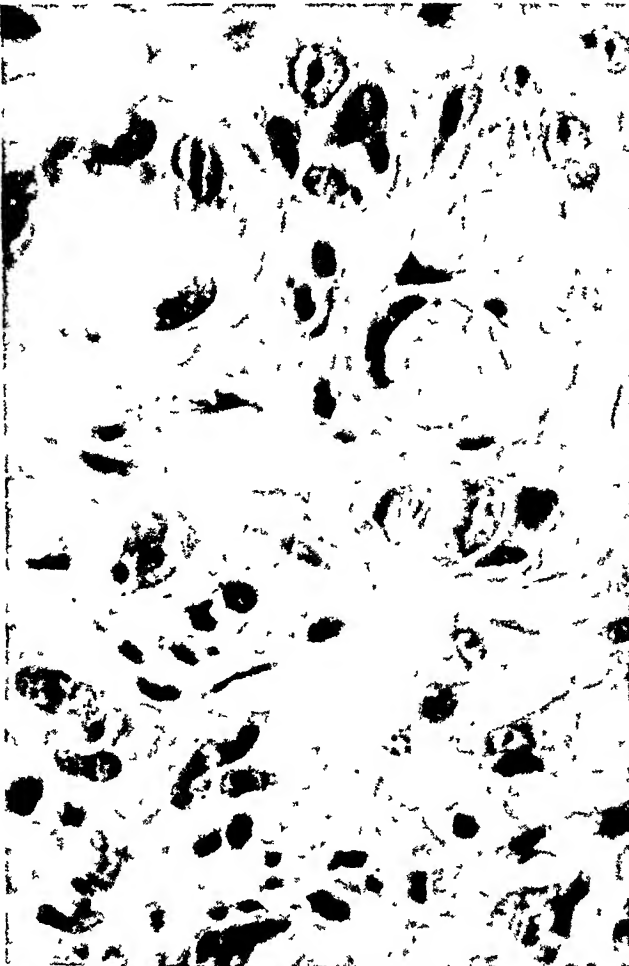
FIG. 7. Giant cells at dermo-epidermal junction and upper dermis. Hematoxylin and eosin stain. $\times 550$.

FIG. 8. Giant cells in the infiltrating portion of a juvenile melanoma. Hematoxylin and eosin stain. $\times 550$.

FIG. 9. Predominantly spindle cell tumor in a male, 20 months old. (From the same case as Fig. 1.) Hematoxylin and eosin stain. $\times 180$.

FIG. 10. Benign nevus in a child, 9 months of age. Hematoxylin and eosin stain. $\times 180$.

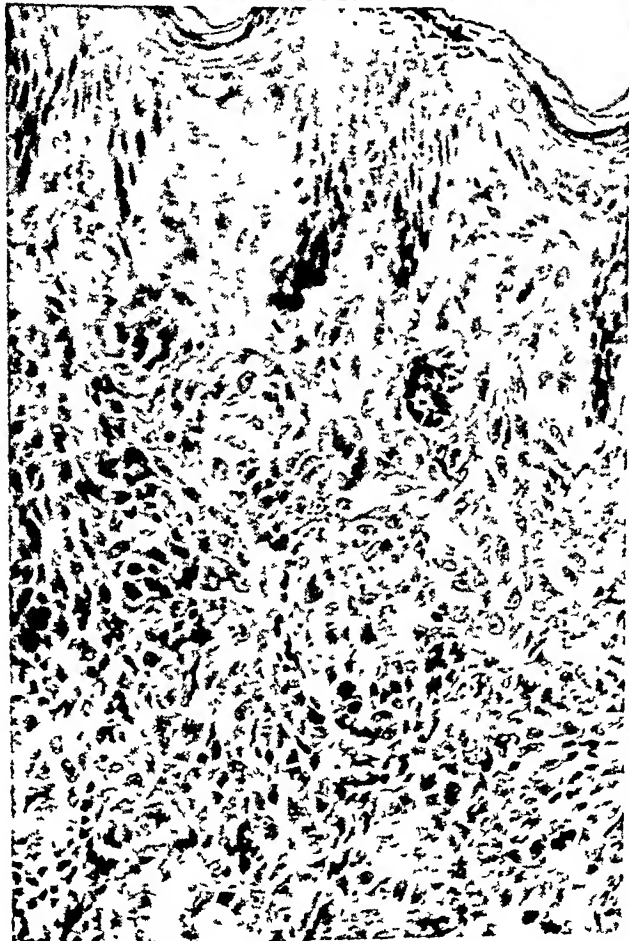
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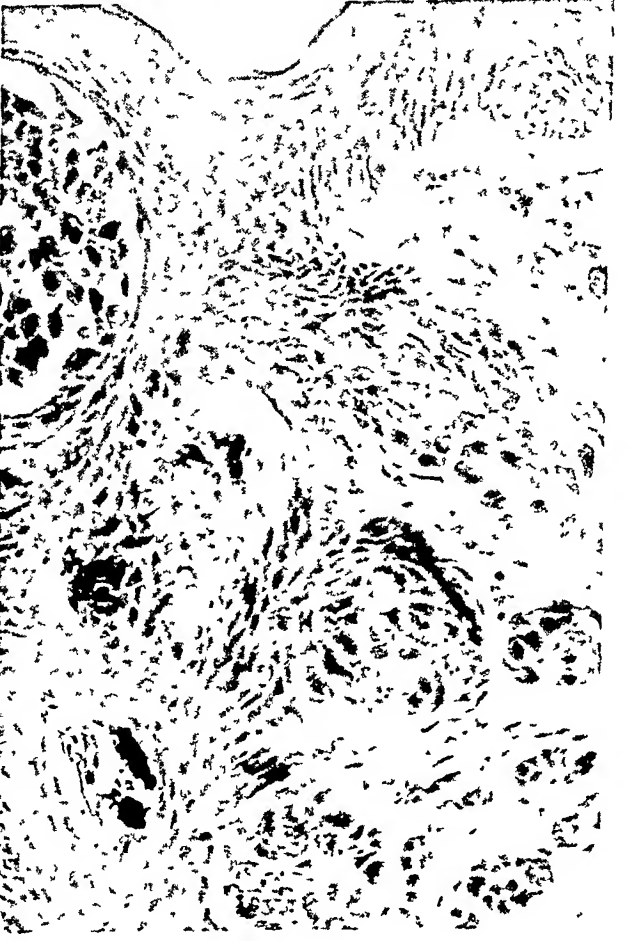
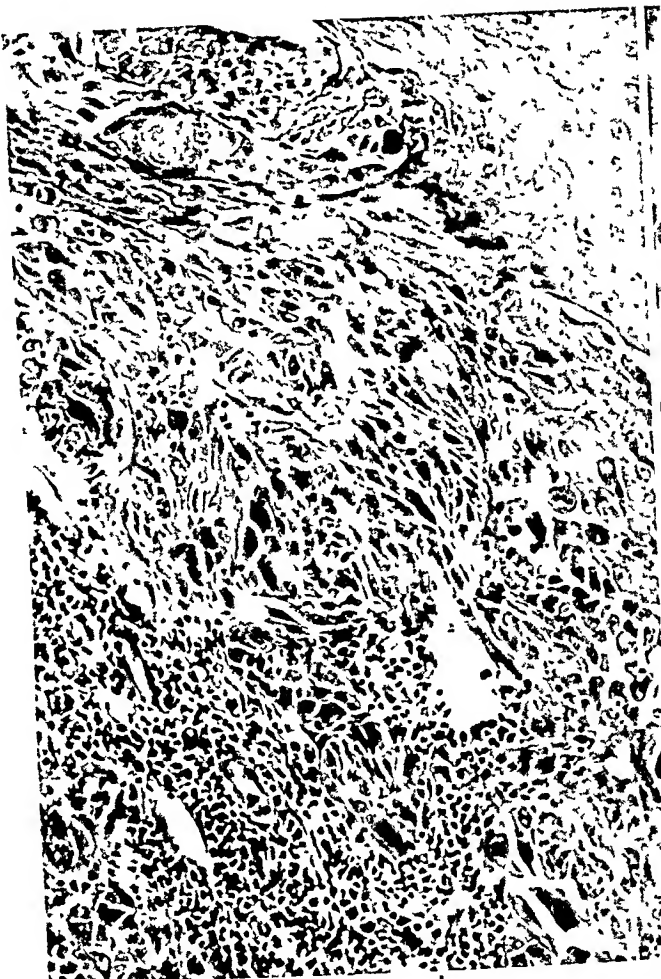


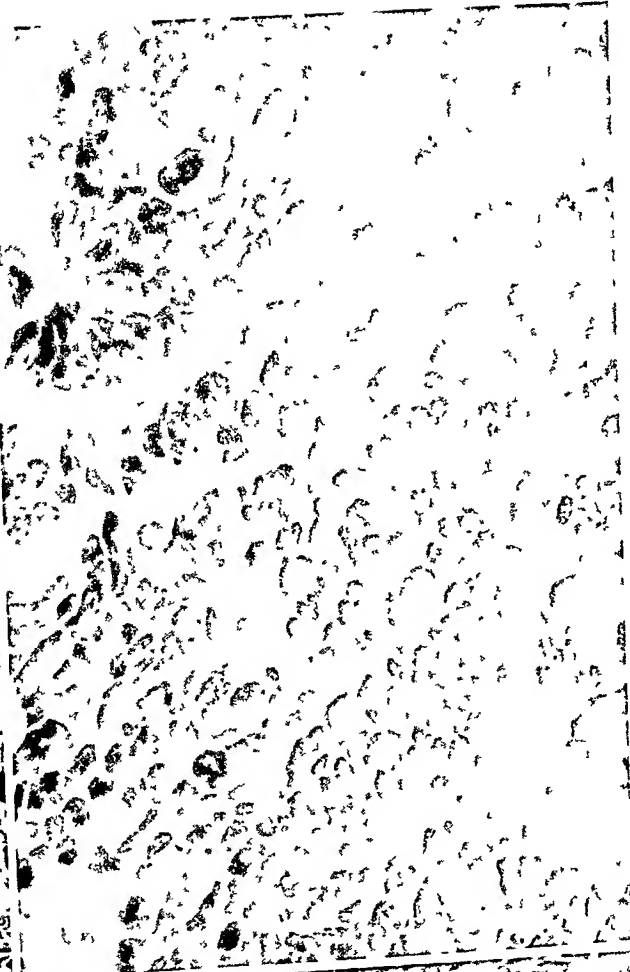
PLATE 110

- FIG. 11. Persistent giant cells in a melanoma of a female, 17 years old. Survival now 5 years. Hematoxylin and eosin stain. $\times 220$.
- FIG. 12. Spindle cell structure in a fatal case of juvenile melanoma (female, 12 years old; death 4 months after local excision). Hematoxylin and eosin stain. $\times 180$.
- FIG. 13. Pleomorphic structure of a clinically malignant juvenile melanoma (case of Dr. Bjarne Pearson). Epidermis in this field has been destroyed by cautery. Hematoxylin and eosin stain. $\times 180$.
- FIG. 14. Rapidly fatal malignant melanoma in a male, 14 years of age. Hematoxylin and eosin stain. $\times 160$.

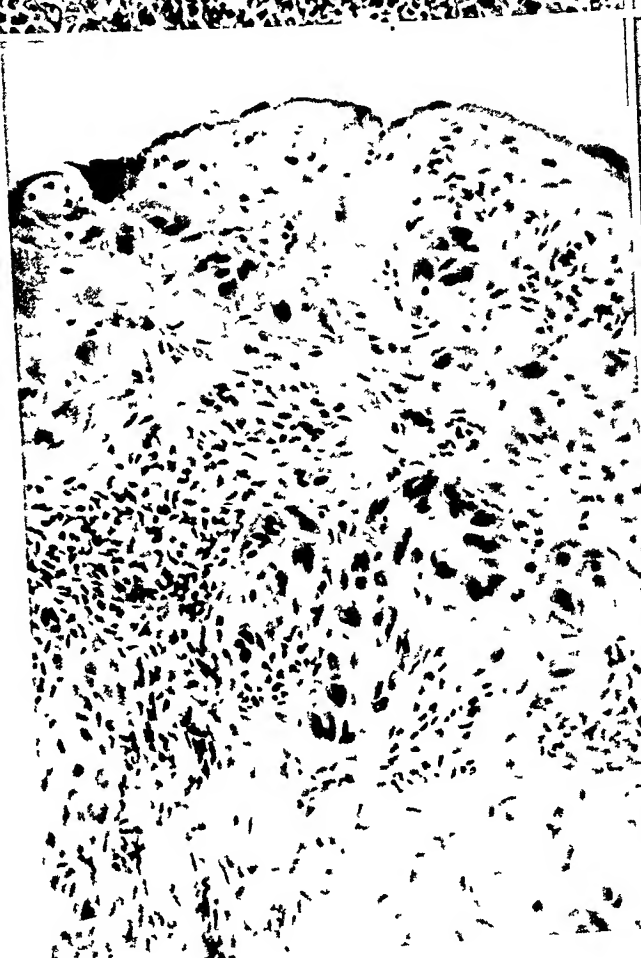
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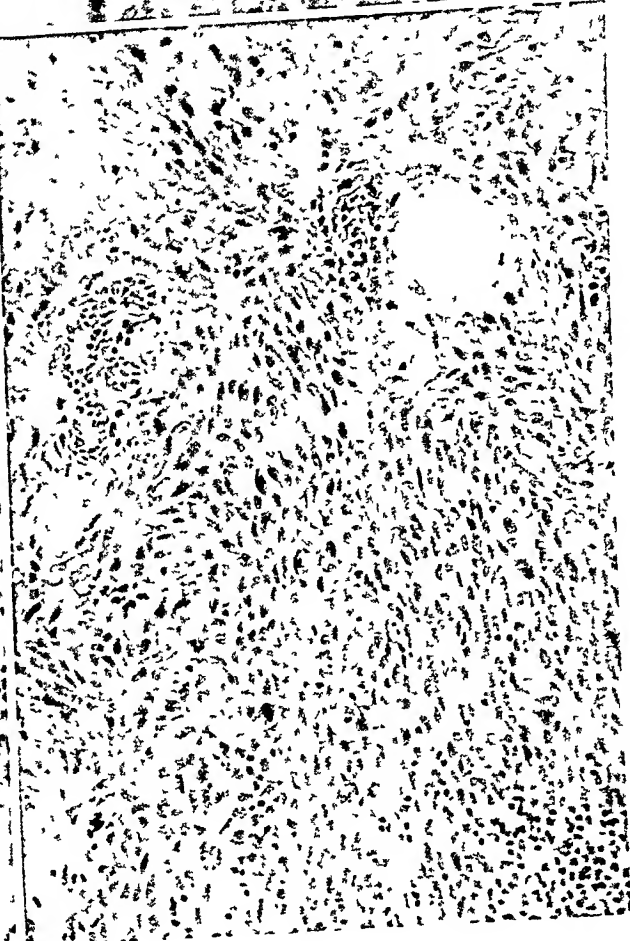
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Melanomas of Childhood

THE LESIONS OF SCHISTOSOMIASIS JAPONICA *

MARK M. BRACKEN, M.D., WILLIAM R. BAILEY, JR., M.D., and
HENRY M. THOMAS, JR., M.D.

(From the Departments of Pathology and Medicine, University of Pittsburgh School of Medicine, the Pathological Laboratories, Mercy Hospital, Pittsburgh, Pa., and the Department of Medicine, Johns Hopkins University Medical School and Hospital, Baltimore, Md.)

During the early days of the occupation of the Philippine Islands in October and November, 1944, some of the American troops were unavoidably exposed to water infested with *Schistosoma japonicum*. The clinical picture of acute schistosomiasis japonica in this epidemic has been discussed by Thomas and Gage,¹ Billings *et al.*,² Winkler *et al.*,³ Tillman,⁴ and others. Description of the lesions of the acute stage has heretofore been lacking, except for depiction of human material by Ash and Spitz,⁵ and experimental observations in animals infested with *Schistosoma* reported by Fairley,⁶ Hoeppli,⁷ and Kopisch.⁸ Although death rarely occurs in the acute stage of schistosomiasis japonica, three of the soldiers exposed in the Philippine Islands died and were autopsied at overseas United States Army hospitals.⁹ Unfortunately, because of the local situation of the hospitals performing the post-mortem examinations, gross specimens were not preserved. Consequently, the complete picture in some of the organs is not available. In addition to material from the three cases studied at autopsy, tissue was secured for biopsy from acute lesions in the rectum, liver, and skin from other patients. Still later and unexpectedly, opportunity was afforded to secure material obtained for biopsy from cerebral lesions in American Army personnel who had returned to the United States. The older lesions of the disease, seen in three Filipinos who died in American Army hospitals following gunshot wounds, are included for comparison.†

The life history of *S. japonicum* has been described by Craig and Faust¹⁰ and more recently by Faust.¹¹ The ova are passed in the feces of infected man or domesticated animals and, on reaching fresh water, hatch within a few hours. The liberated miracidia penetrate the snail host (*Oncomelania quadrasi* in Leyte, P.I.) where first and

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† In preparation of this paper the following cases were studied: Army Institute of Pathology accession nos. 132699, 136057, 136056, 127286, 123276, 131466, 158928, 140914, 142999, 169042, 146469, 151096, 166305, 152989, 141886, 141575, 153128, 163485, 153127, 167247, 135996, 138331, 142999, 158928. Other aspects of nos. 141886, 141575, 138331, and 142999 have been reported by other authors elsewhere.

second generation sporocysts develop. After about 8 weeks cercariae emerge in great numbers. These adhere to the skin of man or animals at the level of the water surface and, as the skin dries, they burrow through the epidermis in the course of 8 to 10 minutes and enter a lymphatic vessel or venule. In this stage these larvae shed their tails and are known as metacercariae or schistosomulae. After traversing the systemic circulation for a few days, they collect in the small hepatic radicles of the portal veins where they mature in 4 weeks, mate, and for the next 5 to 15 years move around as pairs of adult flukes in the mesenteric veins, with the females depositing eggs in the smaller venules of the large and small intestine and appendix. A large number of ova are swept by the blood stream into the liver and a smaller number by way of the inferior vena cava to the lungs.

Ova also find their way to many other localities in the body. In the acute cases in this series, ova have been found in the mesenteric lymph nodes, skin, brain, meninges, and adrenal medulla. Ova have been demonstrated also in the late cases in retroperitoneal tissues, kidney, cerebellum, and medulla oblongata. In addition, lesions identical to those in which ova were demonstrated but in which the eggs were absent from the sections examined were present in the myocardium.

Although the lesions may vary depending on the structure of the tissue or organ involved, their similarity is to be emphasized. The early lesions are usually miliary, appearing as yellowish white, caseous nodules measuring from 0.5 to 10 mm. in diameter. Microscopically, in some of the lesions there is a necrotic zone around viable ova, as is shown in a section of a mesenteric lymph node (Fig. 1). This necrotic zone is surrounded by eosinophilic leukocytes and fewer neutrophilic leukocytes. The slightly older lesions present central ova, either viable or degenerated, with varying degrees of distortion and calcification, and surrounding epithelioid cells and fibroblastic proliferation in a richly vascular zone. Figure 2 demonstrates this stage in a lesion in the liver. Eosinophilic leukocytes decrease in number as the lesion progresses in age, and lymphocytes then predominate. Frequently the ovum is partially or completely surrounded by multinucleated giant cells. The latter are usually of the foreign body type, but they may have the appearance of giant cells of the Langhans' type, as seen in the brain in Figure 3.

The earliest lesions may coalesce to form large, irregular areas of necrosis in which are scattered the schistosome ova. This coalescent lesion is seen most often in the brain (Fig. 4), but it has been encountered also in the mesenteric lymph nodes. Frequently the early lesions

in the intestinal wall and liver assume the form of small abscesses containing the ova. It is not uncommon to find more than one ovum, even as many as ten, at the center of a lesion in the liver, mesenteric lymph nodes, or retroperitoneal connective tissue.

The only frequent reaction noted in the walls of blood vessels has been endothelial swelling in the capillaries, which may markedly narrow the lumina of the vessels. In the older lesions, perivascular cuffing by lymphocytes has been seen in the brain and liver. No lesion of the periarteritis nodosa type was seen in any of the cases.

Although the lesions produced by the ova of *S. japonicum* run a fairly characteristic course, they may vary depending on the organ involved. Those in the intestine occurred most frequently in the large bowel, but they were found also in the ileum in one early case, and in the ileum and duodenum in a late case. Johnson and Berry¹² have reported on the sigmoidoscopic picture in early cases. The miliary deposits of ova in the rectal mucosa gave the surface a coarsely granular appearance. Slight visible mucosal congestion occurred, but no ulcerations were seen by these investigators. Biopsies of the rectal wall in two patients in the early stages revealed ova in the mucosa and submucosa. In the biopsy of such a lesion the viable ova incite a granulomatous reaction (Fig. 5). A central necrotic zone may surround the ova, epithelioid cells are present, and the peripheral cellular reaction consists almost entirely of eosinophilic leukocytes. In the later cases the lesions in the intestine show a moderate amount of fibrosis, and the eosinophils are replaced by lymphocytes. One section of the duodenum of a late case demonstrated ova passing through the mucosal epithelium to enter the lumen of the bowel (Fig. 6). The appendix was involved in several cases and showed a reaction similar to that seen in the rest of the intestine.

The lesions in the liver (Fig. 2) follow the pattern seen in the intestine. However, as the disease progresses, the compact fibrosis which is so characteristic of the later stages spreads along the portal radicles to produce the picture of "pipestem" cirrhosis described by Stitt¹³ in his textbook on tropical diseases. In the early stages, endothelial swelling has been seen in the central veins of the lobules, and portal thrombosis was noted only in one case. Late cases showed hemosiderin-like pigment in the Kupffer cells.

The lesions in the lungs tend to remain discrete rather than to become confluent, and serial sections of late lesions showed that they remain as nodules rather than developing fibrosis along the course of the pulmonary vessels. However, the lung of one case in the acute

stage showed an interstitial pneumonitis. Numerous eosinophils were present throughout the sections. A few ova were demonstrated in the sections of this lung.

Mesenteric lymph nodes of acute cases revealed hyperplasia of the germinal centers of the follicles. The sinusoids were filled with lymphocytes, plasma cells, and a few eosinophilic leukocytes. In one acute case showing ova in the lymph nodes, the ova were engulfed by giant cells of the Langhans' type and the surrounding tissue was necrotic. In advanced lesions the ova were present in the center of dense fibrous tissue nodules. Pigment similar to that seen in the liver was present in a relatively small number of reticulo-endothelial cells in the late cases.

Although the intestine, liver, and lungs are the most frequent sites of involvement, other organs may be involved as has been noted previously. Several patients who had returned to the United States were later operated upon because of various symptoms of disease of the central nervous system, and biopsy of the cerebral lesions revealed ova of *S. japonicum*. Vitug *et al.*,¹⁴ Carroll,¹⁵ Tillman,⁴ and others have reported on the cerebral involvement in this disease. In these diffuse lesions a soggy cortex and medulla were produced. Small discrete lesions frequently were not recognizable grossly. Microscopically, necrosis was widespread (Fig. 4). Eosinophils were present in moderate numbers, but small round cells, including both lymphocytes and plasma cells, sometimes predominated at the periphery of the necrotic zone. Swelling of the capillary endothelium in the region of the lesions was a fairly constant finding. Perivascular cuffing by lymphocytes occurred in the late cases. The acute and advanced lesions otherwise resemble the classical "pseudotubercle" seen in other organs. In the relatively early cases ova have been found in the cerebral medulla of the temporal lobe, middle and posterior superior temporal gyri, parietal lobe, and right occipital lobe. Examination of a late case disclosed ova in the medulla and in the cerebellar cortex.

Biopsy of a nodule in the skin of the abdominal wall, one of a chain of such nodules following the course of an intercostal blood vessel (described by Fishbon¹⁶), revealed the acute lesion of schistosomiasis japonica, with a centrally placed ovum. An arteriole in the section showed focal endothelial swelling. The vessel was surrounded by hemorrhage.

A very extensive myomalacia cordis was present in the left ventricle in one acute case of schistosomiasis. Fibrinous pericarditis overlaid the involved myocardium and there was a large mural thrombus. Sec-

tions showed widespread necrosis and degeneration of myocardial fibers. There was marked fibroblastic proliferation and cellular infiltration of plasma cells and lymphocytes, with fewer neutrophilic and eosinophilic leukocytes. Arterioles and venules in the degenerated area presented hyalinoid necrosis without cellular infiltration of their walls. There was early organization of the inflamed pericardium. The mural thrombus contained many leukocytes of which the greater number were eosinophils, but was without organization. The cause of the infarction was not apparent, for ova were not demonstrated in the heart or pericardium. The coronary blood vessels were not available for further study. In a late case showing myocardial involvement, the lesion was localized to a small area heavily infiltrated by lymphocytes. At the center of the lesion there was a foreign body giant cell. The ovum which undoubtedly produced the lesion was not seen in the several sections examined.

One of the acute cases disclosed ova in the adrenal medulla. An eosinophilic cellular infiltration surrounded them.

A late case presented a focal area of necrosis in the pituitary gland, but ova and cellular reaction were absent.

The genitourinary system showed nothing of note in the acute cases. In one late case an ovum was found in the afferent arteriole of a glomerulus. Slight lymphocytic cellular infiltration had occurred around this glomerulus.

Passive congestion and slight enlargement were the only notable findings in the spleen in the acute cases. In the late cases pigment similar to that seen in the Kupffer cells in the liver appeared in the reticulo-endothelial cells. Of the three late cases the largest spleen weighed 340 gm. and the smallest, 135 gm.

Extreme fibrosis of retroperitoneal tissues was present in one late case, a female with unilateral edema in the left leg. Ova of *S. japonicum* were present singly and in clusters in the fibrous tissue. The latter was relatively vascular and lightly infiltrated by lymphocytes.

SUMMARY

In tracing the lesions of schistosomiasis japonica from the early to the advanced stages, the disease in man is seen to bear a marked similarity to the lesions of schistosomiasis produced experimentally in animals. The general appearance of the lesion varies somewhat, depending on the organ involved, but as a rule it follows a fairly uniform course. When the ovum enters the tissues an extensive cellular reaction occurs, and this consists chiefly of eosinophilic leukocytes, with

fewer neutrophilic leukocytes. In some of the lesions necrosis of tissue occurs in a fairly wide zone around the ovum. Epithelioid cells appear and multinucleated giant cells engulf the ovum. The inflammatory cellular response changes to one in which lymphocytes and plasma cells are most numerous. Fibroblastic and capillary proliferation begin early in the peripheral zone, and, as the lesion advances in age, fibrosis predominates. The oldest lesions consist of shrunken, calcified ova surrounded by more or less dense fibrous tissue, with moderate lymphocytic cellular infiltration. In this pathologic picture of the disease the early lesions represent an unusual and characteristic reaction to the viable ovum with necrosis and eosinophils, and the later lesions represent a foreign body reaction.

We gratefully acknowledge the cooperation of the Army Institute of Pathology which furnished much of the material for examination and permitted the use of the photomicrographs reproduced with this paper.

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[Illustrations follow]

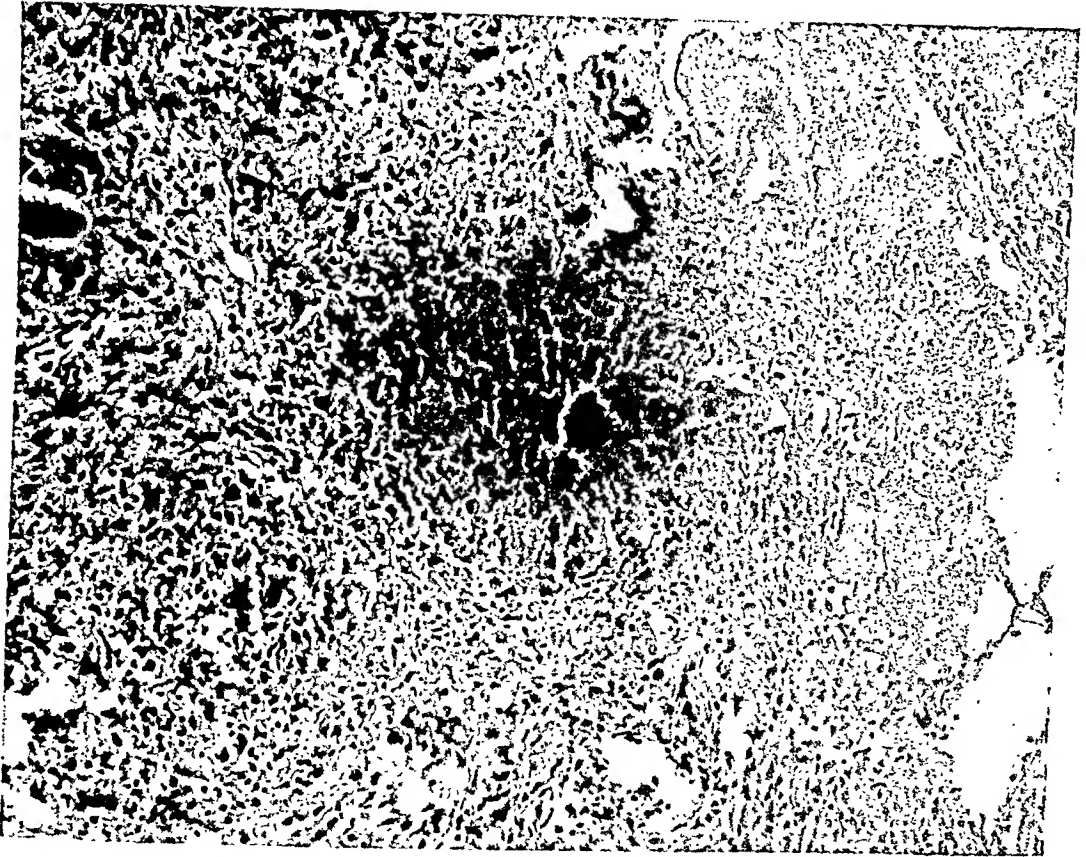
DESCRIPTION OF PLATES

PLATE III

FIG. 1. Necrotic zone surrounding an ovum in a lymph node. $\times 145$. (Army Institute of Pathology negative no. 97398.)

FIG. 2. Viable ovum, surrounded by necrosis and numerous eosinophils in the liver. $\times 175$. (A.I.P. neg. 97396.)

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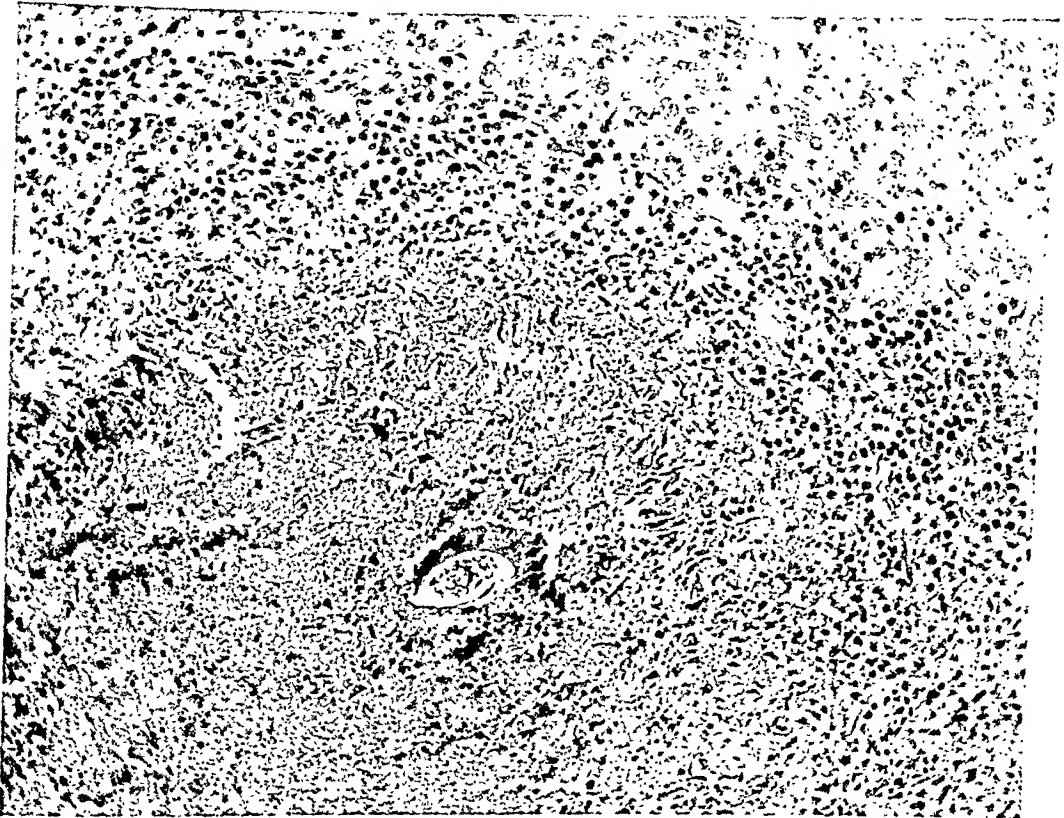


PLATE 112

FIG. 3. Lesion in cerebral medulla, with one distorted ovum, multinucleated giant cells, epithelioid cells, and leukocytes. $\times 120$. (A.I.P. neg. 93480.)

FIG. 4. Coalescing lesions in the brain. $\times 20$. (A.I.P. neg. 43469.)

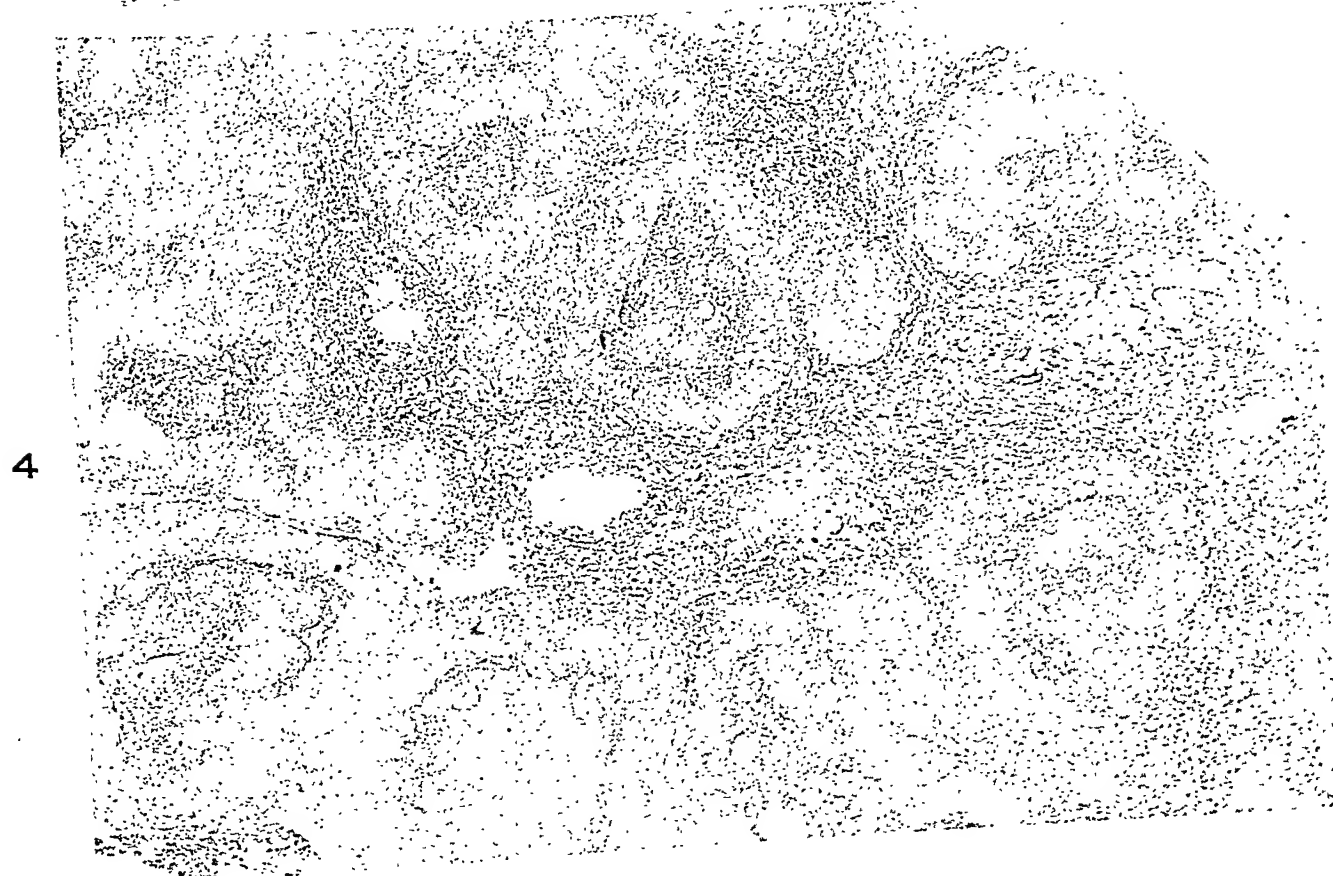
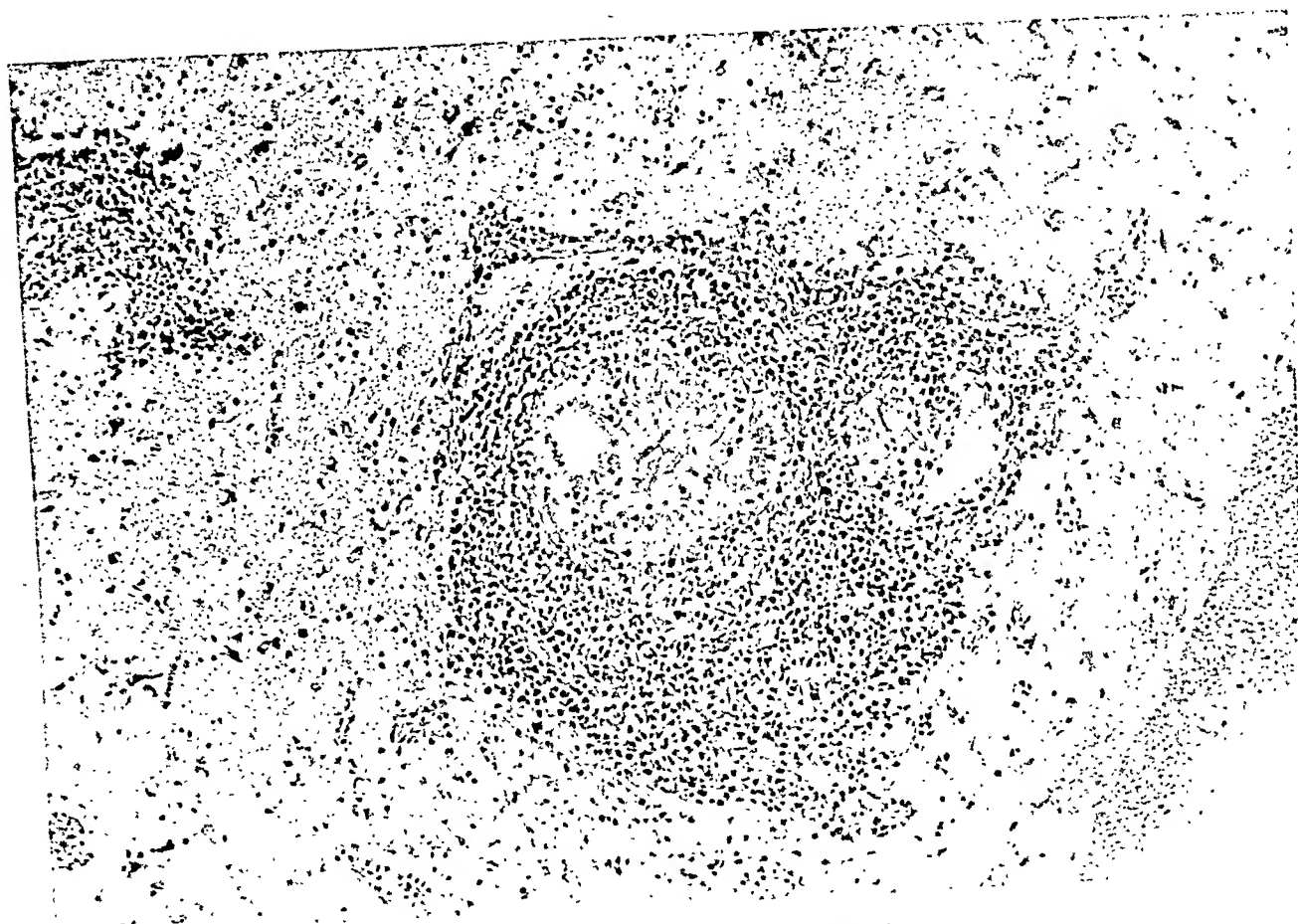
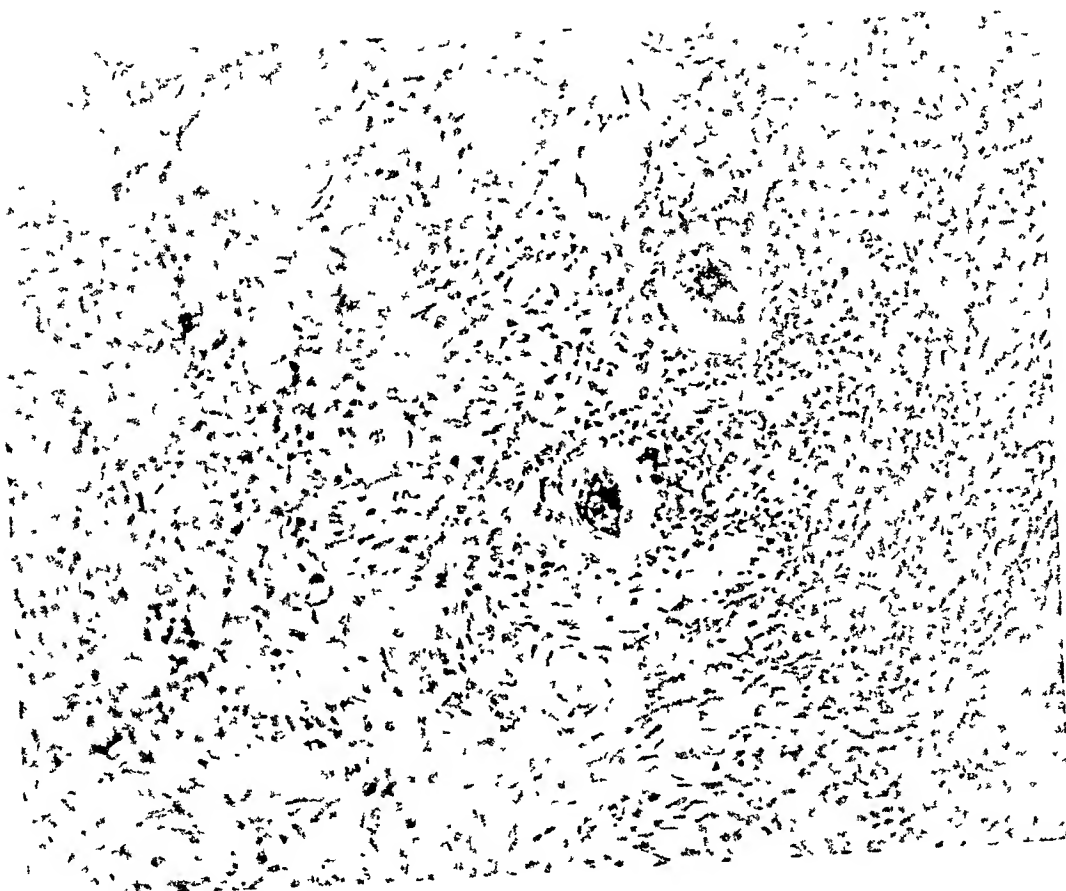


PLATE 113

FIG. 5. Viable ova in rectal mucosa. $\times 210$. (A.I.P. neg. 97389.)

FIG. 6. Ova being extruded into the lumen of the duodenum. $\times 145$. (A.I.P. neg. 97385.)

5



6



Schistosomiasis Japonica

Bracken, Bailey, and Thomas

THE SIGNIFICANCE OF LOCAL VASCULAR PHENOMENA IN THE PRODUCTION OF ISCHEMIC NECROSIS IN SKELETAL MUSCLE*

JOHN W. HARMAN, M.B.†

(From the Department of Pathology, University of Wisconsin Medical School, Madison 6, Wis.)

The occurrence of a peculiar form of degeneration induced by ischemia in skeletal muscle has been described in a previous publication.¹ It was considered to be identical with Bowman's discoid change, which consists of transverse fragmentation of skeletal muscle fibers into broad anisotropic disks. The degeneration is an invariable result of prolonged, unrelieved ischemia, and has been conclusively associated with the pathogenesis of ischemic necrosis of muscle by several observers, both experimentally¹⁻³ and clinically.^{4,5} From experimental studies it is apparent that the disks first appeared subsequent to unrelieved ischemia of 4 hours' duration, and thereafter increased until they were nearly ubiquitous at 18 hours. Since all fibers so affected were non-viable,¹ their rate of development was considered to be a satisfactory index of the rate of necrosis of the fibers. It was further found that release of the tourniquet after 4 hours of ischemia did not preclude extension of discoid degeneration, which was as considerable after 18 hours as that associated with a similar period of unrelieved ischemia. Because of this observation it is apposite to inquire more extensively into the vascular phenomena associated with the pathogenesis of acute and chronic ischemic necrosis of skeletal muscle. Although occlusion of major vessels may initiate the process, other factors appear to operate toward perpetuation of the ischemia when the external pressure is relieved.

The rôle played by the vessels in the pathogenesis of ischemic necrosis of skeletal muscle was first evaluated in the classical clinical descriptions by Volkmann⁴ and his pupil, Leser,² who ascribed the necrosis of muscle to the ischemia induced by compression of the arteries with constrictive dressings. However, the steady accumulation of cases in which dressings could not be implicated unsettled this view, which was finally replaced by the hypothesis that necrosis was caused by venous obstruction due to a local hematoma,⁶ an opinion shared by Brooks' from experimental studies.⁷ This assumption remained undisturbed until it was clearly shown by Griffiths⁸ that the lesions caused by venous and arterial occlusion are distinct, and that

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† Dr. Denis J. Coffey (Traveling Fellow in Physiology, University College, Dublin (National University of Ireland)).

the former in no way resemble those of ischemic necrosis. On the basis of his experience with arterial embolism and direct trauma to arteries, he advanced the opinion that the ischemia was due to arterial spasm, a point of view supported by Barnes and Trueta⁹ and Leriche,¹⁰ so that culpability came to rest again upon the arteries.

It is noteworthy, however, that no consideration had been given to the capillaries. Except for the work of Wertheimer, Dechaume, and Frieh,¹¹ who plugged the venules by retrograde injection of a gelatin mass, which is virtually capillary obstruction, no study of these vessels in relation to ischemic necrosis has been uncovered. Yet any assessment of the vascular phenomena peculiar to ischemia must take the intimate vasculature into consideration. This is particularly necessary in skeletal muscle because of the unique gradient of permeability in muscle capillaries¹² and the consequences which disturbances of the gradient may produce in the tissue.

METHOD

Complete ischemia was produced in the right hind legs of albino male rabbits weighing 2,000 to 3,000 gm. The animals, which were permitted food and water freely, were first narcotized by an intraperitoneal injection of 35 mg. of "veterinary" nembutal per kg., usually in a volume of 5 cc. Ischemia was then induced in the right hind leg by the application of tourniquets, such as were used for rats by LePage,¹³ or by the use of a tightly wound rubber band (Eberhard Faber no. 64), which was applied according to the method of Rosenthal.¹⁴ These were adjusted so as to encircle the thigh about 0.5 cm. proximal to the knee, because with higher obstruction the animals frequently died in shock. Complete arterial occlusion was confirmed by lack of swelling in the ischemic leg as well as by the failure of fluorescein to pass distal to the tourniquet when it was injected intravenously in amounts as great as 5.0 cc., and the leg was inspected under an ultraviolet lamp screened to exclude all except light of 4,000 Å.¹⁵ Preference was given to the rubber band method as a means of producing ischemia because of the instantaneity of application and release which it permitted. The obstruction was removed at the end of the period of ischemia. The great saphenous artery was sought by palpation, and its pulsation identified before further procedures were instituted. It could be rolled against the tibia and felt to pulsate vigorously.

Histologic studies were made on formalin-fixed tissue, prepared as paraffin sections, cut to a thickness of 7 μ , and stained with hematoxylin and eosin. Although a more extensive histologic analysis is in prep-

aration, the general histologic features of the diseased tissues will be described, in so far as they have a bearing upon the problem immediately under investigation.

EXPERIMENTAL PROCEDURES

Investigation of Large Arteries and Veins

Angiography was carried out on animals subjected to ischemia for from 4 to 4½ hours with a release of obstruction for 3, 24, and 48 hours thereafter. Each animal was anesthetized with nembutal and, when required, with ether. A laparotomy was performed and the abdominal aorta was dissected free of its surrounding tissue. With the animal under the x-ray apparatus, a 20 gauge needle was inserted into the vessel and thorotrast (Heyden Chemical Co., 24 to 26 per cent ThO₂ by volume) slowly injected without interference with blood flow. After 5.0 cc. had been injected, an exposure was made (factors 50 ma, 50 mv, at 36 inches distance and for ¾ second). When a further final 5.0 cc. had been introduced, a second exposure was made, and a third made 1 minute subsequent to completion of the injection. In several animals sodium iodide, made up as a 20 per cent solution in isotonic sodium chloride, was used, but because of the violent muscle spasm, hyperpnea, and convulsions attendant upon its injection it was not used routinely.

In a few animals a 20 per cent (by volume) solution of Higgins' India ink was injected in a similar manner to that employed for thorotrast, in amounts equivalent to 3.0 cc. per kg. The needle was held in the aorta so that blood flow was not impeded during infusion; thus the injections were comparable to those with thorotrast. Within a few minutes the animal was sacrificed and both extensor and flexor muscles excised from the ischemic and normal legs. Tissue from both groups was then fixed in 10 per cent formalin and subsequently cleared by the method of Spalteholz, to permit direct visualization of the injected vessels.

Irrespective of the duration of the ischemia, it was possible immediately after release of the tourniquet to palpate the pulse in the great saphenous artery. Subsequently, with the accumulation of edema fluid, it was often difficult to detect the pulse unless the fluid was well pressed aside digitally, when it was usually readily accessible. In the few instances when palpation was equivocal, the skin was reflected and pulsation determined by direct visualization. The angiographic studies in 10 instances (Table I) confirmed this patency, and are worthy of further interpretation. The first roentgenogram (Fig. 1) is an example

of a first exposure, in which it is seen that not only were the arteries patent and well filled, but that those on the right were more dilated than the normal vessels. In Figure 2, which represents the second exposure, the veins had begun to fill on the normal side whereas those on the ischemic side were not visualized. In the third roentgenogram (Fig. 3) the venous filling on the left or normal side was less, and that on the right more apparent. This sequence was repeated in all 10 cases. From this it may be inferred that the arteries and veins were patent, but that there was a slowing of the circulation in the smaller vessels of the tissues between the arteries and veins. There was no especial indication that the thorotrast passed through the muscles, for

TABLE I
Angiography (by Thorotrast) of Limb Ischemia (4½ Hours)

Number of experiments	Interval between release of tourniquet and x-ray	Radio-opacity of vessels		Pulse*
		Arteries	Veins	
5	hours 1½-4	+	+	+
3	24	+	+	+
2	48	+	+	+

* Pulse determined by palpation of great saphenous artery.

Factors in roentgenography were: 50 kv, 50 ma, ¾ second, and 36 inches.

it might well have circumvented the muscles by shunting through the skin and subcutaneous tissue. However, in the India ink preparations (Fig. 4) particles were observed in the small intramuscular arteries, although the finer ramifications were not so conspicuous as in the contralateral normal muscles. The filling of these small vessels within the tissue indicates that the thorotrast may take a similar path, since its particles are comparable in size to those of the ink.

Investigation of Penetration of Dye

Bromphenol blue (tetrabromophenolsulfonphthalein) was the dye used in studies of the rate of penetration into, and elimination from, ischemic muscle of small, diffusible molecules. It was first used in biologic research by Rous and Drury¹⁶ in their investigations upon graded permeability of fine vessels in rabbit skin. They used a 4 per cent solution, but a 2 per cent solution was used here and injected into the right ear vein in amounts equivalent to 3.0 cc. per kg. The animals so injected were divided into two groups. In one series the dye was

injected within 5 minutes of the release of the tourniquet. Animals were then selected at intervals of $\frac{1}{2}$, 3, and 20 hours, anesthetized with nembutal, and the skin of both hind legs reflected so that direct inspection of the muscles was feasible. The intensities of impregnation of the dye in the ischemic and normal muscles were compared and classified as 0 to 4 plus. In the other group of animals injection of the dye was delayed for 20 hours, after which they were treated in a manner similar to those of the first group. In both groups, when the in-

TABLE II
Passage of Bromphenol Blue (2 Per Cent) through Muscles

Immediate dye injection (5 min.)*			
Ischemia	Before observation	Normal muscle	Ischemic muscle
hours	hours		
2	3 $3\frac{1}{4}$ 20	o o o	\pm o o
3	$\frac{1}{2}$ 3 20	3+ o o	4+ 2+ o
4	$\frac{1}{2}$ 2 20	3+ o o	4+ 3+ 1+
6	$2\frac{1}{4}$ 20	o o	2+ 1+
8	18 20	o o	2+ 1+

* The dye was injected within 5 minutes after release of the obstruction.

tensity of the dye was judged, the tibialis anticus and extensor digitorum longus muscles were peeled off the anterior aspect of the leg as a group and the plantares muscles excised from the flexor mass.

The injection of bromphenol blue (Table II) immediately subsequent to release of the tourniquet was followed by a rapid intense staining of both normal and ischemic muscles, indicating lack of arterial occlusion and free penetration of dye regardless of the duration of ischemia. When an interval of 3 hours elapsed between the injection of dye and the determination of intensity of staining, a distinct pattern of behavior became apparent. Dye was either eliminated entirely or was only scantily retained by muscles which were ischemic for 2 hours; when elimination was retarded, considerable dye was demonstrable in the plasma, indicating that excretion of dye by the

animal was not rapid. When ischemia had been of 3 or more hours' duration, there was considerable retention of dye by the muscle even 3 hours after injection. Consideration of the fate of the dye 20 hours after its infusion revealed that, with such a time interval, evidence of sluggish elimination was first manifest in muscles ischemic for 4 hours. From these findings it may be seen that circulation through the muscle was extremely sluggish. It was impossible to determine whether

TABLE III
Passage of Bromphenol Blue (2 Per Cent) through Muscles

Delayed dye injection (20 hr.)*			
Ischemia hours	Before observation hours	Normal muscle	Ischemic muscle
2	$\frac{1}{2}$ 3 20	2+ o o	3+ ± o
3	$\frac{1}{4}$ 3 20	3+ ± o	3+ 1+ o
4	$\frac{1}{4}$ 3 26	3+ o o	o 2+ ±
6	$\frac{1}{2}$ 4 20	3+ o o	o 2+ ±
8	$\frac{1}{4}$ 1 3½	3+ 3+ ±	o o 2+

* Dye was injected 20 hours subsequent to release of the obstruction.

this slow intramuscular circulation was due to impairment of flow within the muscle or to deviation of blood to other structures; the fact that the dye was eliminated from the skin more rapidly than from muscle would appear to favor the latter view, whereas it might signify merely a relatively greater resistance of skin vessels to ischemia as compared with muscle vessels.

When the injection of dye was deferred until 20 hours had elapsed after release of the tourniquet, the behavior of the dye differed considerably from the preceding pattern (Table III). With ischemia of 2 and 3 hours, respectively, the dye entered quickly and was rapidly eliminated; where elimination appeared retarded, it was found that the plasma was still laden with dye and that the normal muscle had still retained a trace, so that the apparent deficiency of elimination was not significant. After ischemia of 4 to 6 hours' duration, a striking de-

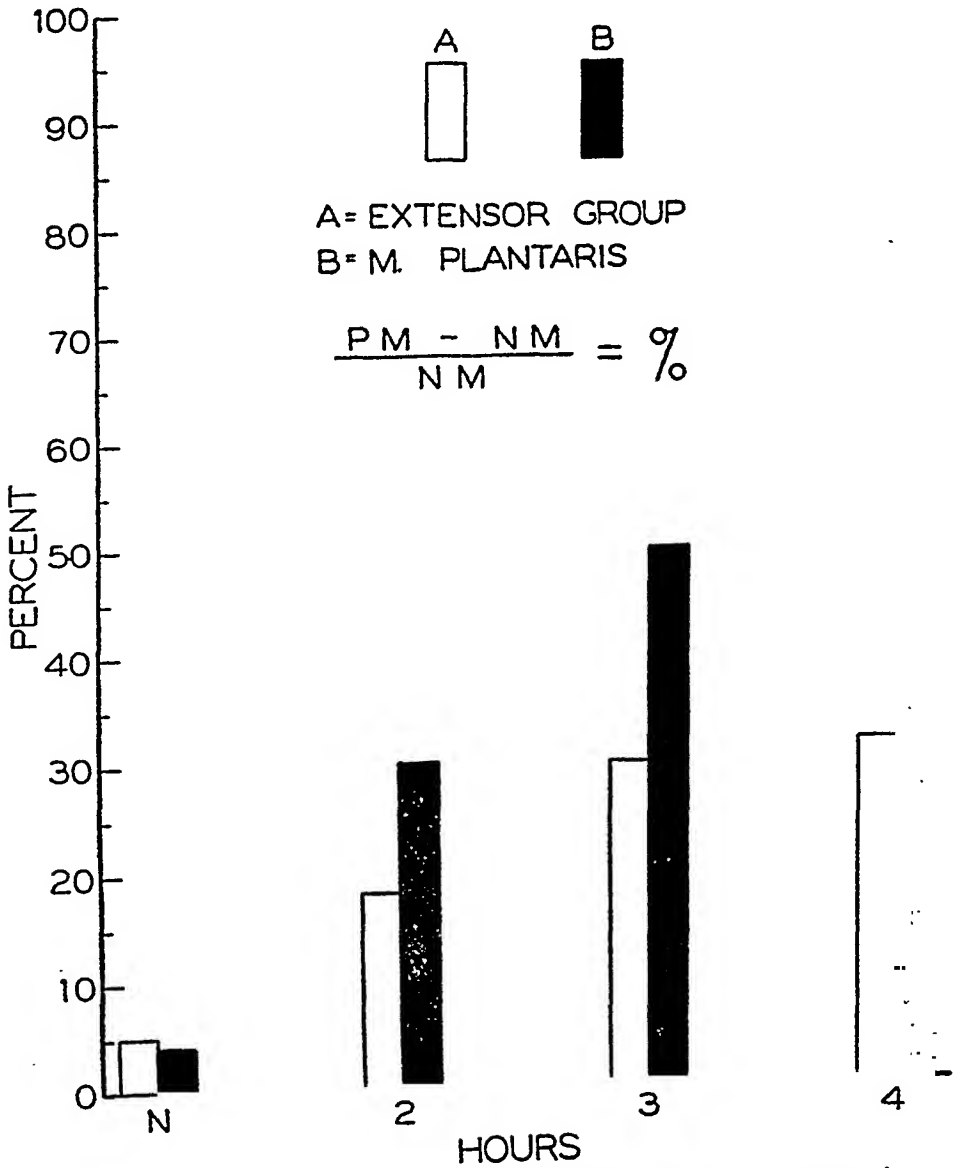
parture was observed in that the dye failed to enter the ischemic muscle for at least $\frac{1}{2}$ hour subsequent to injection, whereas the contralateral muscles were stained to their usual intensity. But 3 hours later the abnormal muscles were deeply stained, when the normal muscle had entirely lost its coloration, so that the circulation was still open, although very slow. In the muscles ischemic for 8 hours no dye had entered even after 1 hour, yet penetration was considerable in a little over 3 hours. It was apparent that with ischemia of 4 hours or longer, and despite a return of blood flow, the circulation re-established through the muscle was abnormally sluggish. On the other hand there was never any delay in staining of the skin comparable to that seen in the muscle.

The histologic picture of the muscles several hours after release of vascular obstruction was complicated by a profuse infiltration of monocytes and polymorphonuclear leukocytes, considerable edema, and extensive degeneration of the muscle fibers. Many fibers were split up into Bowman's disks, some were fragmented into minute, irregular, granular, basophilic particles, and a few had a pale, eosinophilic, waxy appearance; of the several forms of degeneration the Bowman type was predominant (Fig. 5). In these muscles the capillaries appeared ubiquitous, dilated, and engorged with tightly packed erythrocytes, which appeared square because of the pressure due to mutual contiguity. They contained no thrombi or cell-free segments, and extravasations from them were rare and minute. No alterations were observed in the structure or contents of the larger arterial and venous channels.

Assessment of Local Fluid Accumulation

The excised groups of muscles, both ischemic and normal, were in each instance stripped of fascia, pressed dry between filter papers, and weighed rapidly to the nearest 0.01 gm. In a series of normal animals, the difference in weight between the extensor group and the plantaris of the right leg and these muscles in the left was determined to ascertain the normal variation, using the left leg muscles as the standard, and expressing the difference in weights as a percentage of these muscles. Since the right leg usually was rendered ischemic, the increase in weight of its muscles was also expressed as a percentage of the left in a similar manner. It was expected that this would adequately indicate any trend or significant deviation in weight as readily as comparing differences in ratios of wet and dry weight, since it would also be necessary to assume in the latter method that the loss of necrotic tissue by flushing out was balanced by plasma proteins brought in with the edema fluid. It has been stated by MacFarlane and Spooner¹⁷

that the maximum increase in weight due to edema is reached within ½ hour for guinea-pigs, and by Koletsky and Gustafson¹⁸ that it is reached in 3 hours in rats after release of ischemic obstruction to a limb. Since the ischemic muscles weighed in this series were all released for 3 hours or longer, it may be assumed that all had attained their maximum degree of edema.



Text-Figure 1. The duration of ischemia is plotted against percentage increase in weight. PM = weight of ischemic muscle. NM = weight of normal muscle. Each column represents the average of ten or more estimations. Column N signifies the average maximum increase between corresponding normal muscle in a series of untreated controls.

The average percentile difference in weight between similar muscles in the leg, those in the left leg being used as standard, is plus or minus 3 per cent. The average weight difference of the ischemic muscles expressed in a similar manner is demonstrated in Text-Figure 1; in

which the increase falls far outside the normal range of variation between similar muscle groups. It was striking that there was considerable edema after even 2 hours of ischemia, but that the difference between this and the edema following 3 hours of ischemia was marked. On the other hand, the edema after 3 and 4 hours of ischemia was not significantly different. It was further apparent that the extent of edema in the plantaris muscle, a representative of the flexor group, was invariably much greater than that of the extensor group as represented by the tibialis anticus and extensor digitorum longus, a fact which must be accounted for in determining the importance of edema *per se* in abetting the effects of ischemia.

Development of Chronic Lesions

In a further set of experiments, the animal was not disturbed after release of the tourniquet, except to examine the pulse and estimate the extent of edema and paralysis, until a period of 10 to 30 days had

TABLE IV
Relation of Duration of Ischemia to Development of Chronic Lesions

Number of Experiments	Ischemia	Duration of experiment	Infarcts	Average change of weight*
	<i>hours</i>	<i>days</i>		
4	3	10-30	0	-30%
5	4	10-24	2	-30% (No infarct)
				+50% (Infarcted)
4	6	12-15	4	+50%
2	8	15-16	2	+30%
2	12	10-18	2	+80%

* All muscles which increased in weight contained infarcts; those without infarcts were decreased in weight.

elapsed. At the elected time the animal was anesthetized and injected with 4 per cent bromphenol blue to stain the tissues intensely. The leg muscles were then exposed after $\frac{1}{2}$ hour, a period adequate to allow staining of normal tissue. The presence of gross areas of necrosis was determined in the muscles; such areas were avascular and consequently free of dye. The muscles were then excised and treated similarly to the previous groups.

In the light of the previous findings, the late sequelae of varied periods of ischemia of skeletal muscle were very significant (Table IV). The muscles which were ischemic for less than 4 hours were shrunken and firm, elastic and contractile, and stained very deeply with the injected dye (Fig. 6). When the difference in weight was

expressed as a percentage of the weight of the normal muscle, it was found that the weight of the ischemic muscle was significantly decreased, although no gross lesions were observed. When ischemia had lasted for 4 hours, some muscles resembled those subjected to 3 hours of ischemia; others contained large, greenish yellow, depressed, hard, friable areas, sharply demarcated from the ends of the muscle (Fig. 7) and the surrounding normal tissue. The yellow areas were not contractile, although the parts surrounding them were electrically irritable. Such areas of necrosis occurred in all tibialis anticus and plantaris muscles ischemic for longer than 4 hours, and less commonly involved the gastrocnemii. They tended to include the whole muscle except for a small portion adjacent to the tendinous attachments. It was noteworthy that the weights were much increased in those muscles which contained infarcts.

Microscopically, the infarcted areas were composed entirely of fibers which were segmented into anisotropic Bowman's disks or conchoidal plates and which contained no nuclei (Fig. 8). The disks were not seen in the surrounding normal muscle, which was separated from the infarct by a zone of richly vascular, fibroblastic connective tissue in which there were occasional multinucleated muscle cells. The most conspicuous result from this series was that it indicated the occurrence or completion of some phenomenon at the end of 4 hours of ischemia, because of which the character of the final muscle damage was altered from a diffuse moderate wasting caused by the shorter periods to a severe massive necrosis with impairment of function.

DISCUSSION

From the histologic character of the areas of infarction or "muscle sequestra," as Griffiths⁸ termed them, the lesions caused by temporary arterial occlusion are identifiable with those found in experimental ischemic necrosis due to unrelieved arterial occlusion.^{1,3} This lesion is comprised of a central mass of yellow necrotic tissue, made up of fibers segmented into Bowman's disks, around which is a zone of fibroblastic connective tissue, the vascularity of which is considerable. This differs from the lesion caused by venous occlusion, in which there is no sequestrum, but merely a sparse scattering of atrophic muscle fibers widely separated by dense fibrous tissue,¹⁹ a picture never seen with arterial occlusion. Furthermore, the experimental lesions are identical with those found in Volkmann's contracture. From this histologic evidence it is possible to infer that venous obstruction plays no part in the pathogenesis of experimental and clinical ischemic necrosis of

skeletal muscle; this is substantiated by the free venous filling observed in the roentgenograms.

The involvement of the large arteries also may be excluded because of the invariably palpable pulse along the great saphenous artery, which is an index of the patency of large arteries below the site of obstruction. The reliability of this criterion as an index of adequate arterial patency is dependent upon the finding of Mann *et al.*,²⁰ that it is necessary to reduce the lumen of a vessel to 12 per cent of its normal area before the blood flow to the area beyond the obstruction is nearly halved. In all instances the easy palpation of the pulse indicated an expansion of the arterial lumen compatible with normal flow, since it is known that even after 50 per cent reduction in size of the lumen there is no appreciable effect upon rate of flow. This patency is amply confirmed by the angiographic studies which revealed not only patency but an actual dilatation of the arterial tree in the distal part of the previously occluded limb. The discrepancy of this finding with those of Barnes and Trueta⁹ may be due to the difference in radio-opaque medium used. They employed a solution of sodium iodide, which was highly irritating and which induced severe muscle spasm. I had similar experience with it, and adopted the use of thorotrast, which is non-irritant and has a neutral reaction. With the latter medium, spasm was never encountered. While these experiments cannot exculpate arterial spasm entirely as an occasional cause of muscle necrosis, they exclude it as the common mechanism arising from temporary arterial occlusion, such as Griffiths,⁸ Barnes and Trueta,⁹ and Leriche¹⁰ suggest.

While it is irrefutable that arterial occlusion initiates the process of ischemic necrosis, it is equally certain that some other cause than persistent arterial obstruction must be sought to explain the perpetuation of the damage so inaugurated. The behavior of bromphenol blue in its slow penetration into, and tardy elimination from, the muscle indicates that the fault lies within the muscle itself. That this behavior of the dye is caused by adsorption to the proteins of inflammatory exudate appears improbable because it has been shown by Miller²¹ under different experimental conditions that this particular dye was absorbed more rapidly from inflamed than from normal tissue. The slow entrance of the dye would favor the contention, however, that the blood is shunted away from the muscle and into the skin, which is usually rapidly tinted. The mechanism of this would be by sympathetic paralysis, which, as Siddons²² and Cohen²³ pointed out, causes a deviation of blood flow into the skin at the expense of the muscles. And it must

be recalled that ischemic contracture is almost invariably accompanied by nerve injury.²⁴ Consequently, it is not possible to discount entirely this variation in circulation on the present evidence. Caution is further implemented by the finding that in man, intra-arterial adrenalin infusion causes vasodilatation in the muscles of the leg,²⁵ followed by vasoconstriction, independently of sympathetic action.

On the other hand, this is not the decisive factor because a profound paralysis is induced by ischemia of 2 and 3 hours, after which infarction always has been absent. This being so, it is probable that the cause of the sluggish flow through the damaged muscle lies within the tissue and is of such a nature as to impede the inflow of arterial blood. Since the veins are not blocked, the site of the obstacle must be in or around the venules, capillaries, and fine arterioles. Histologically, these minute vessels are silted up with red cells, which signifies that the liquid component of the blood has filtered off almost entirely and left behind a vessel plugged with conglutinated particulate elements. This is the picture of stasis invoked by severe capillary damage.^{26, 27} Because of this capillary damage, stasis, and the obliteration of the normal gradient of permeability,¹² there is a considerable resistance to the inflow of blood, and considerable depression of the rate of exchange of metabolites, of which bromphenol blue may be a reliable criterion since its diffusion coefficient is not far removed from that of dextrose. The difference in the degree and type of muscle damage caused by periods of ischemia of less than 4 hours as compared with those of longer periods is perhaps to be explained by a peculiar vulnerability of the fine vessels to different durations of ischemia, for, though damage apparently follows the shorter periods of ischemia as indicated by edema, it may be irreversible only after the longer. It must be remembered also that, although considerable interstitial fluid accumulates after release of occlusion, judged by weight this is as great after 3 as after 4 hours of ischemia, so that if diffusion through the increased volume of fluid is invoked as a factor in the impairment of metabolic exchange,¹⁹ it could not explain the difference in reversibility between these two periods. Furthermore, the increase in weight of the plantaris muscle is as great after 2 hours as is the increase of the extensor group after 3 or 4 hours of ischemia, although infarction does not appear in the plantaris until after 4 hours of ischemia. Therefore, one would suspect this difference to be due to direct damage to the capillary wall by the ischemia.

It is pertinent to draw attention to the studies by Meneely and co-workers²⁸ on the results of temporary occlusion of the coronary artery in a Bailey-LaDue preparation, which demonstrates progressive dam-

age to the myocardium, as revealed by electrocardiography, after occlusion is released and blood flow re-established. They believed that this is the result of increased capillary permeability to trypan blue. Their conclusion concerning the pathogenesis of ischemic necrosis in cardiac muscle is essentially similar to that which I have reached on the basis of the studies outlined above.

SUMMARY

1. By means of direct palpation and visualization of pulsation and with supplementary angiography, spasm of arteries is excluded as a major factor in the pathogenesis of ischemic necrosis of skeletal muscle.
2. The angiographic studies and histologic lesions indicate that the condition is not caused by venous obstruction.
3. In view of the manner of movement of the dye bromphenol blue into and out of ischemic muscles and the histologic picture of stasis it is most likely that the principal cause of the ischemic necrosis is the persistence of initial ischemic damage of the intimate vasculature.

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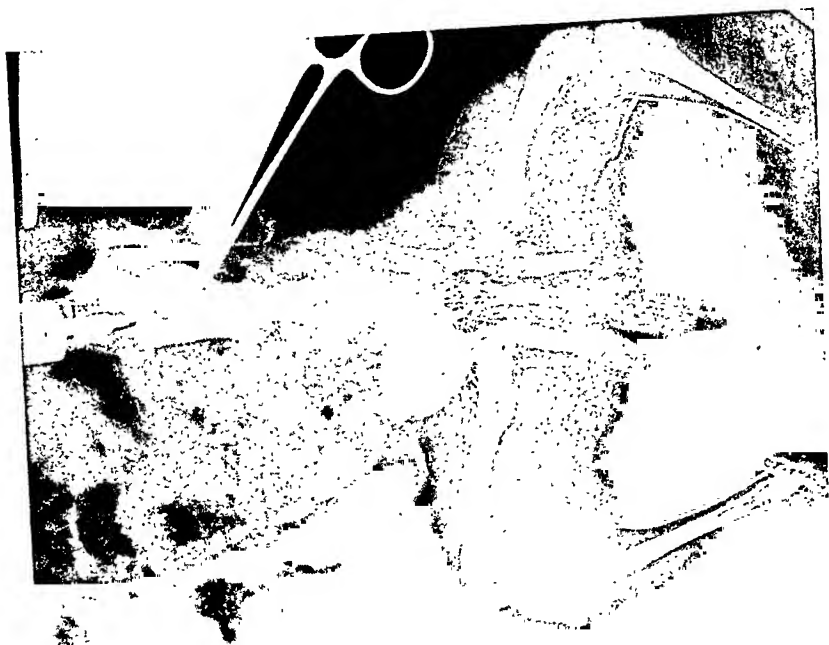
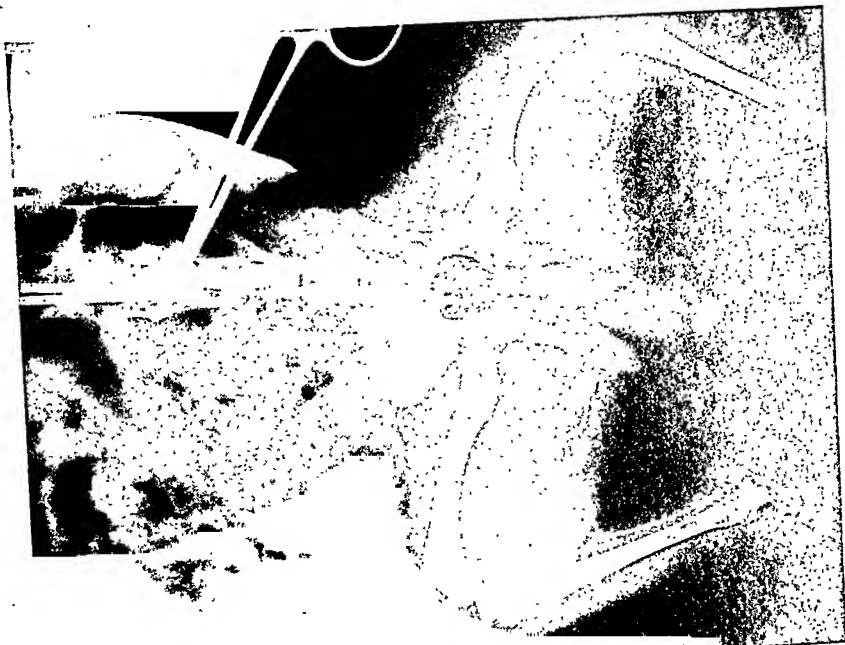
DESCRIPTION OF PLATES

Plate 114

FIG. 1. Thorotrast injection in an animal with ischemia of the right leg for 4½ hours, and subsequent release for 4 hours prior to injection. The roentgenograph was taken when 5 cc. of thorotrast had been injected into the aorta. The arteries of both legs are clearly visualized; there is dilatation of those in the right leg below the site of ischemic obstruction. $\times \frac{1}{4}$.

FIG. 2. Second roentgenograph in the same animal as in Figure 1 after a further and final injection of 5 cc. of thorotrast. The arteries are still conspicuous. The veins are now visualized in the normal left leg. $\times \frac{1}{4}$.

FIG. 3. Roentgenograph taken 30 seconds subsequent to the last injection. The arteries are no longer visualized, but the veins are now clearly outlined in both legs and have commenced to fade in the normal left leg. $\times \frac{1}{4}$.



3

2

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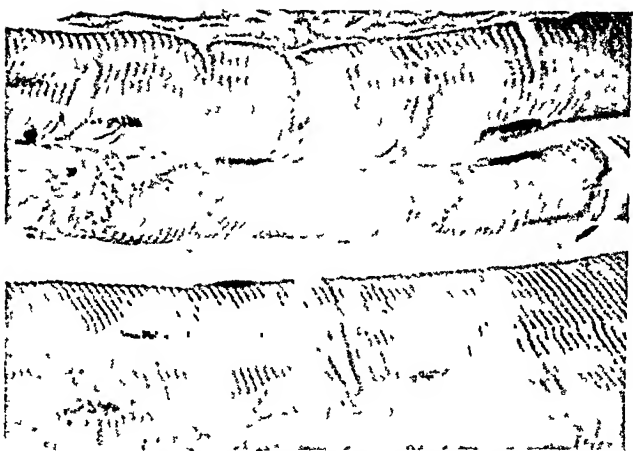
Ischemic Necrosis in Skeletal Muscle

Harman

PLATE 115

- FIG. 4. After India ink is injected into aorta, extensor muscles then are excised and cleared by Spalteholz' method. The small intramuscular arteries are clearly defined by the India ink, down to even minute ramifications in both muscles. Abnormal muscle had been ischemic for 4 hours. $\times \frac{1}{4}$.
- FIG. 5. Fibers from tibialis anticus muscle released for 24 hours subsequent to 4 hours of ischemia. Longitudinal striations are absent. The fibers are split transversely into Bowman's anisotropic disks, are thick and individualized. Nuclei are tigroid due to clearing of nucleoplasm and clumping of chromatin. Hematoxylin and eosin stain. $\times 400$.
- FIG. 6. Extensor muscles from a normal leg (on the right) and from a leg made ischemic for .3 hours with a subsequent recovery period of 21 days. Normal muscle is larger, less firm, and less deeply stained with dye. No infarct was seen in the ischemic muscle. $\times \frac{1}{2}$.
- FIG. 7. Plantares muscles from the legs of an animal which had ischemia of one leg for 8 hours. Released for 18 days before excision. The pathologic muscle consisted entirely of an infarct, except for a small area adjacent to the tendinous insertion. $\times \frac{1}{2}$.
- FIG. 8. Edge of an infarcted area of 30 days' duration. Circulation had been released after ischemia of 6 hours. The infarct is composed of fibers split into Bowman's disks. Surrounding the infarct is a compact zone of fibroblastic connective tissue. Nuclei are absent in the infarcted area. Hematoxylin and eosin stain. $\times 400$.

4



6



THE PERIODIC ACID ROUTINE APPLIED TO THE KIDNEY *

J. F. A. McMANUS, M.D.

(From the Department of Pathology, Medical College of Alabama, Birmingham 5, Ala.)

This communication presents results of a study of the kidney with the aid of an original staining method which has been described previously¹ for the demonstration of mucin (Fig. 1).

In brief, microscopic sections are treated with an aqueous solution of periodic acid and then colored with Schiff's reagent for aldehydes. Schiff's reagent or leuko-basic fuchsin is a straw-colored solution which is produced by the action of sulfurous acid on an aqueous solution of basic fuchsin. The reagent takes on a red or violet color when an aldehyde is added. In tissues it forms a red or violet insoluble compound at the sites where aldehyde is present. This property is utilized in various histochemical technics. In the classical Feulgen's test, the aldehyde which is formed from desoxyribose nucleic acid by hydrolysis with weak hydrochloric acid is colored with Schiff's reagent. In similar fashion, Bauer's test for the demonstration of glycogen makes use of Schiff's reagent, aldehyde being produced by the action of chromic acid.

Since periodic acid has been known to produce an aldehyde when acting upon a carbohydrate² and when acting upon serine, threonine, or hydroxylysine,³ it seemed a natural sequence to test it on tissue sections. It has been reported already that the following materials are colored by Schiff's reagent after the action of periodic acid: mucin in the intestinal and respiratory tracts, the colloid of the pituitary stalk and thyroid, mucous salivary glands, certain cells of the anterior hypophysis, and the basement membrane of the renal tubules and glomeruli.¹ The present communication describes the technic in greater detail, including the preparation of the reagents, and reports further results in the application of the periodic acid Schiff's reagent routine to the kidney.

MATERIALS AND METHODS

The sections of kidney which were studied came from young adult males, killed in the European campaign, and from the autopsies of the Jefferson-Hillman Hospital. The material had been handled in a variety of ways, although most of it had been fixed in formol-saline or in Zenker's-formol solution. A cobalt-calcium-formol fixative⁴ was found useful, particularly when postchromed by leaving for 24 to 48

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hours in 3 per cent dichromate and washing in running tap water for 12 to 24 hours before dehydration. This is the method of choice when the granular cells of the renal arteriole are to be studied. The tissues were dehydrated in alcohol and embedded in paraffin after either toluene or xylene. Some tissues which had been in formaldehyde solution for a long time gave satisfactory appearances after postchroming. It may be noted here that postchroming appeared to improve the histologic and cytologic detail after nearly all fixatives.

The preparation of the necessary reagents is not a complicated matter.

The routine used to prepare Schiff's reagent is as follows:

1. Weigh out 1 gm. of basic fuchsin
2. Weigh out 1 gm. of anhydrous sodium bisulfite
3. Boil 200 cc. of distilled water
4. Add fuchsin and stir
5. Cool to 50° C.
6. Filter
7. Add 20 cc. of normal HCl (98.3 cc. of HCl (sp. gr., 1.16) made up to 1 liter)
8. Cool to 25° C.
9. Add sodium bisulfite

Keep in the dark. The fluid takes 1 or 2 days to become orange, or straw-colored, when it is ready for use.

Sulfurous acid rinse:

- 6 cc. of 10 per cent sodium metabisulfite
- 5 cc. of normal HCl
- 100 cc. of distilled water

These directions are those of Dr. John R. Baker (personal communication) and derived from Lison.⁶

The routine used for coloring the basement membrane is as follows: Paraffin sections cut at 3 to 6 μ are brought through xylene and graded alcohol to water. When the tissue has been hardened in a fixative containing mercury, the customary treatment with 0.5 per cent of I₂ in 70 per cent alcohol and 5 per cent sodium thiosulfate is carried out and the sections are washed for several minutes in running tap water. They are placed in a solution of 0.5 per cent periodic acid for 2 to 5 minutes at room temperature and then washed in distilled water briefly, although long washing in tap and distilled water does not appear to influence their appearance. They are placed for 15 minutes in Schiff's reagent at room temperature. After removal, the sections are treated for 2 minutes in each of three changes of sulfurous acid as in the classical Feulgen's test. Departure from the original technic consists of washing the sections, after the last sulfurous acid rinse, in a staining dish through

which a stream of tap water is running, for 5 to 10 minutes. This has been found to enhance considerably the brilliance of the coloration. The sections may then be brought through graded alcohols to xylene and mounted in balsam or, if a counterstain is desired, they may be placed in Harris' hematoxylin for 30 seconds and then washed thoroughly in a stream of tap water for 5 to 10 minutes before being dehydrated and mounted. If a trichrome effect is desired, the sections, after being washed following the hematoxylin nuclear stain, are placed in 0.1 per cent light green for 15 seconds or less and then washed in water before being dehydrated.

RESULTS

The basement membranes of the glomeruli and of the tubules are colored bright red or purple, as are the cell outlines of the smooth muscle cells of the arterioles and capillary walls. Ordinary connective tissue stains little if at all and there is no nuclear coloring without a counterstain. Elastica is not stained nor are the erythrocytes or the cytoplasm of cells apart from the proximal tubule. The "brush" border of the cells of the first convoluted segment is colored constantly and the cytoplasm of these cells colors to a degree which varies from one nephron to another and from case to case. There is some coloring of the cytoplasm of polymorphonuclear leukocytes and of isolated droplets or granules in various parenchymal cells, frequently in the position of the Golgi element.

Counterstaining colors the chromatin blue to black. Light green stains the cytoplasm of the cells and the erythrocytes, and also the basement membrane to some extent, particularly in the glomerulus. The granules of the afibrillar arteriolar cells in the "crush" kidney are colored a bright red, somewhat lighter in shade than that of the basement membranes. When it is desired to study the arteriolar granules, it is necessary to obtain tissue within 1 hour after death, and the cobalt-calcium-formol fixative seems the best, with postchroming essential. Alcohol-toluene or alcohol-xylene methods of dehydration and clearing must be used.

The normal glomerulus shows a single basement membrane by this technic (Fig. 2). It is believed that glomerular structure can be interpreted more thoroughly with the present technic than in sections prepared by the trichrome stain or by modifications of Mallory's aniline blue-orange G mixture.

In the "crush" kidney there is a strong prominence of the granular cells of the renal arterioles, as Goormaghtigh⁶ has described. The

fibrin of venous thrombi (Fig. 3) stains strongly by this method. The hyalin-appearing casts in the tubules stain with varying degrees of brilliance. Glycogen and amyloid are colored brightly.

The alteration of the basement membrane of the glomerulus in arteriosclerosis, which was described by McGregor,⁷ is made prominent (Fig. 4).

The hyalin of arteriolosclerosis (Fig. 5) shows strong coloring. Hyaline droplets (Fig. 6) in epithelial cells of the proximal tubules color brilliantly. Where tubules have become atrophied in scars, there is persistence of staining of the basement membranes of the tubules except in the oldest scars. It was concluded from the study of many cases that thickening of the basement membrane of the corresponding tubule is an early change which occurs in any progressive glomerular injury.

DISCUSSION

Three features appear to be worthy of discussion at the present time.

The Utility of the Method as a Histologic Aid in the Study of the Kidney

The basement membranes of glomeruli and tubules are so well shown by this technic that it is proposed as the method of choice. Fresh tissues, from autopsies performed within 1 hour after death, are best. When autopsy is delayed, the postchroming in 3 per cent potassium dichromate for 24 hours followed by washing of the tissue in running water for the equivalent time produces satisfactory results.

A particular advantage of the method is that no "differentiation" of the section is necessary. This is in contrast to the aniline blue-orange G and Masson's trichrome technics in which the end result is a personal technical production. With them, differences in results may be produced by minor variations in the length of differentiation or of staining and by minor variations in the quality of the dye. Serial sections cut at quite long intervals of time give identical results with the periodic acid routine.

The Structure of the Normal Glomerular Basement Membrane

The glomerulus in the normal kidney shows a single basement membrane by the present technic. The glomerular alterations in a variety of diseases which have been studied appear to be capable of explanation upon this basis. Nothing is revealed with any certainty as to the nature of the basement membrane of the glomerulus or of the tubules. It is of interest in connection with the pathogenesis of Bright's disease

and other affections of the kidney that a coloring similar to that seen in the basement membrane is observed in the hyalin of arteriolo-sclerosis, in casts, and in hyaline droplets.

The Histochemical Validity of the Present Method

When the periodic acid technic was described originally, it was pointed out that it was of histologic rather than of histochemical usefulness. That is still the present position. Malaprade² had introduced periodic acid into quantitative chemistry, having found that it would produce an aldehyde when it acted upon the connection between two carbon atoms of a chain if each of these two adjoining carbon atoms had a hydroxyl group. Nicolet and Shinn³ found that the bond between adjoining carbon atoms was broken and that an aldehyde was formed when one carbon atom had a hydroxyl group and the other an amino group. The demonstration of aldehyde in tissue sections by Schiff's reagent after the action of periodic acid was a logical attempt, but histochemical conclusions are difficult to reach because of the following two facts. Lison⁵ has shown that a great variety of materials other than aldehydes, notably ketones and unsaturated compounds, will recolor Schiff's reagent. Secondly, many substances in tissues contain one or both of the linkages from which periodic acid can produce aldehyde. Separate identification of glycogen, glycoprotein, glycolipid, and the three amino acids (serine, threonine, hydroxylysine) is necessary if the method described is to give valid histochemical data. It is my opinion that the basement membrane consists of a carbohydrate-protein compound of the mucoprotein type, but decision must be deferred for the reasons given.

SUMMARY AND CONCLUSIONS

In sections of normal human kidneys the basement membranes, capillary walls, and the outlines of the smooth muscle cells of the arterioles are colored with Schiff's reagent after periodic acid.

Besides these structures, the same routine applied to abnormal kidneys colors the following materials: The hyalin of arteriolo-sclerosis, hyaline casts, glycogen, amyloid, colloid droplets in tubular epithelium, and the granules of the afibrillar cells of the arterioles.

The material of the basement membrane which takes the stain is believed to be a mucoprotein. Opposed to immediate acceptance of this hypothesis are (1) the nonspecificity of the recoloring of Schiff's reagent, and (2) the production of aldehyde by periodic acid from three amino acids.

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DESCRIPTION OF PLATES

All illustrations were made from sections treated with periodic acid followed by Schiff's reagent.

PLATE 116

FIG. 1. Human jejunum. There is coloring only of mucous goblets and of the free surface ("brush border") of the intestinal epithelium. $\times 1500$.

FIG. 2. Normal glomerulus and arteriole from a white female, 42 years of age. The basement membrane of the glomeruli and tubules is colored as well as the cell outlines in the arteriole. There is slight coloring of the free surface of the proximal convoluted tubules. $\times 520$.



PLATE 117

FIG. 3. Tubular injury and venous thrombosis after a severe wound. A thrombus can be seen protruding into the venule from the inflamed interstitial tissue. There is a hyaline cast in one tubule and some cellular debris in another. Counterstained with hematoxylin. $\times 320$.

FIG. 4. Glomerulus from a case of essential hypertension in a colored female, 36 years old. Death in second cerebral hemorrhage. Most of the glomeruli, like the one of which a corner is shown, appeared normal; a few—one-fourth to one-fifth—showed the wrinkling of the simplified basement membrane which is illustrated. Hyaline arterioles (not shown) were numerous. $\times 520$.

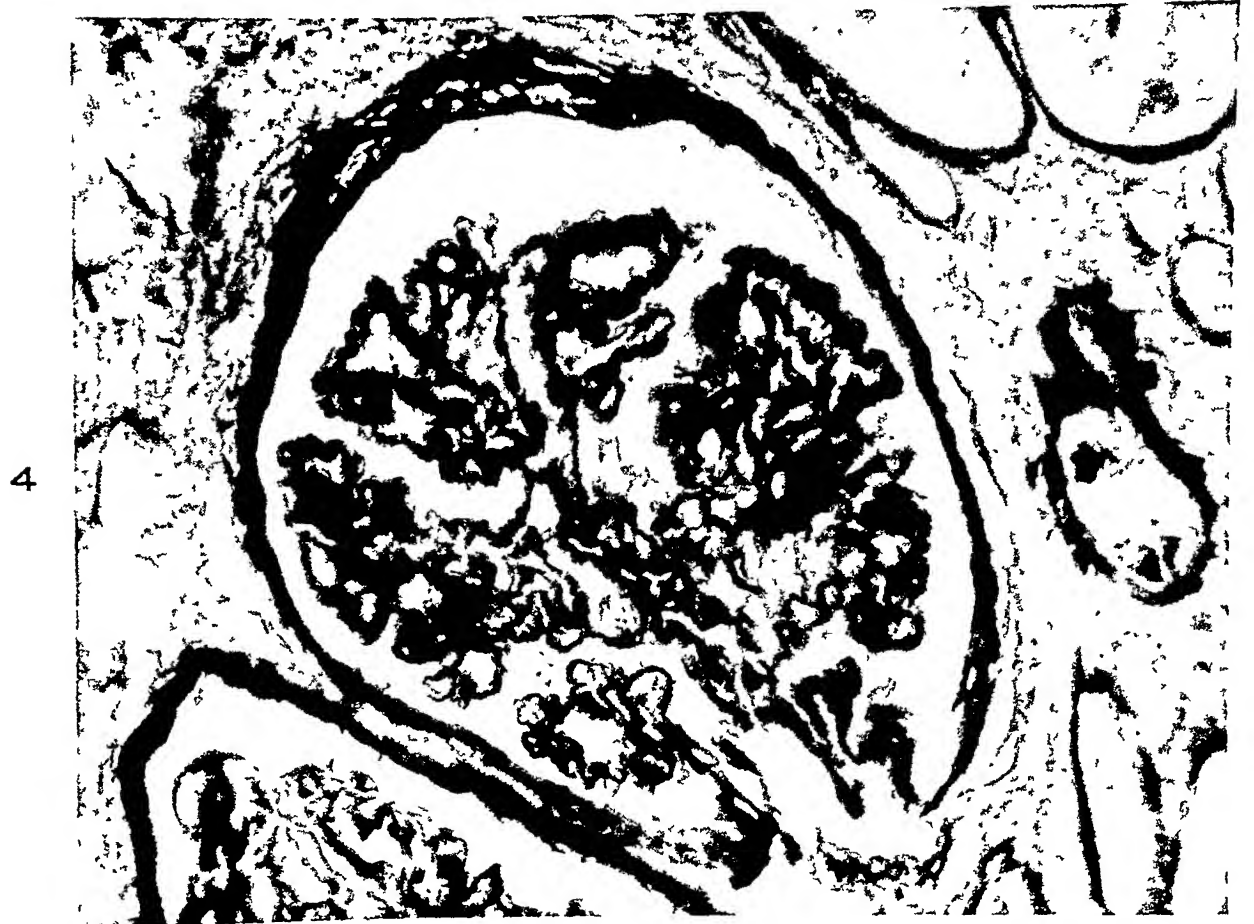
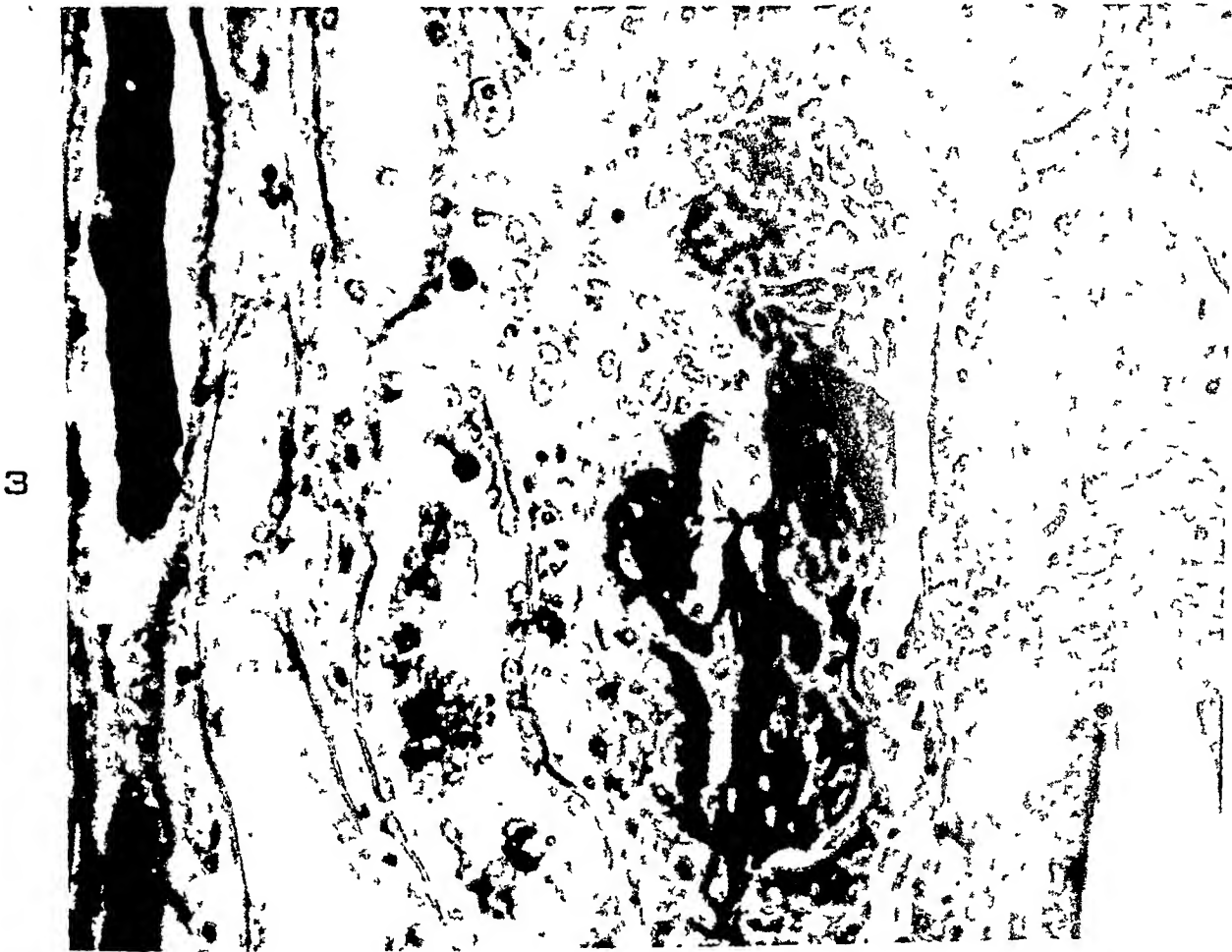
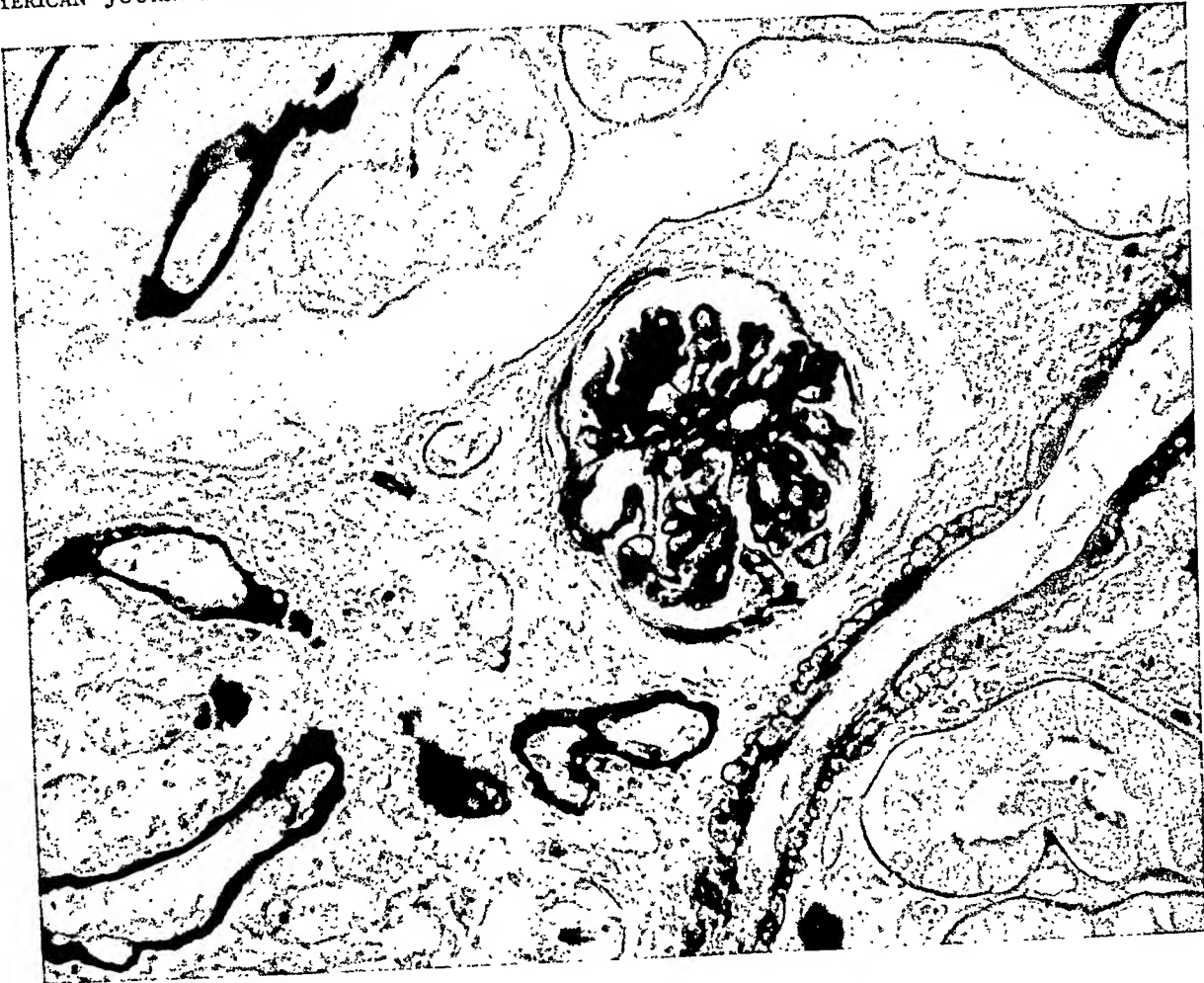


PLATE 118

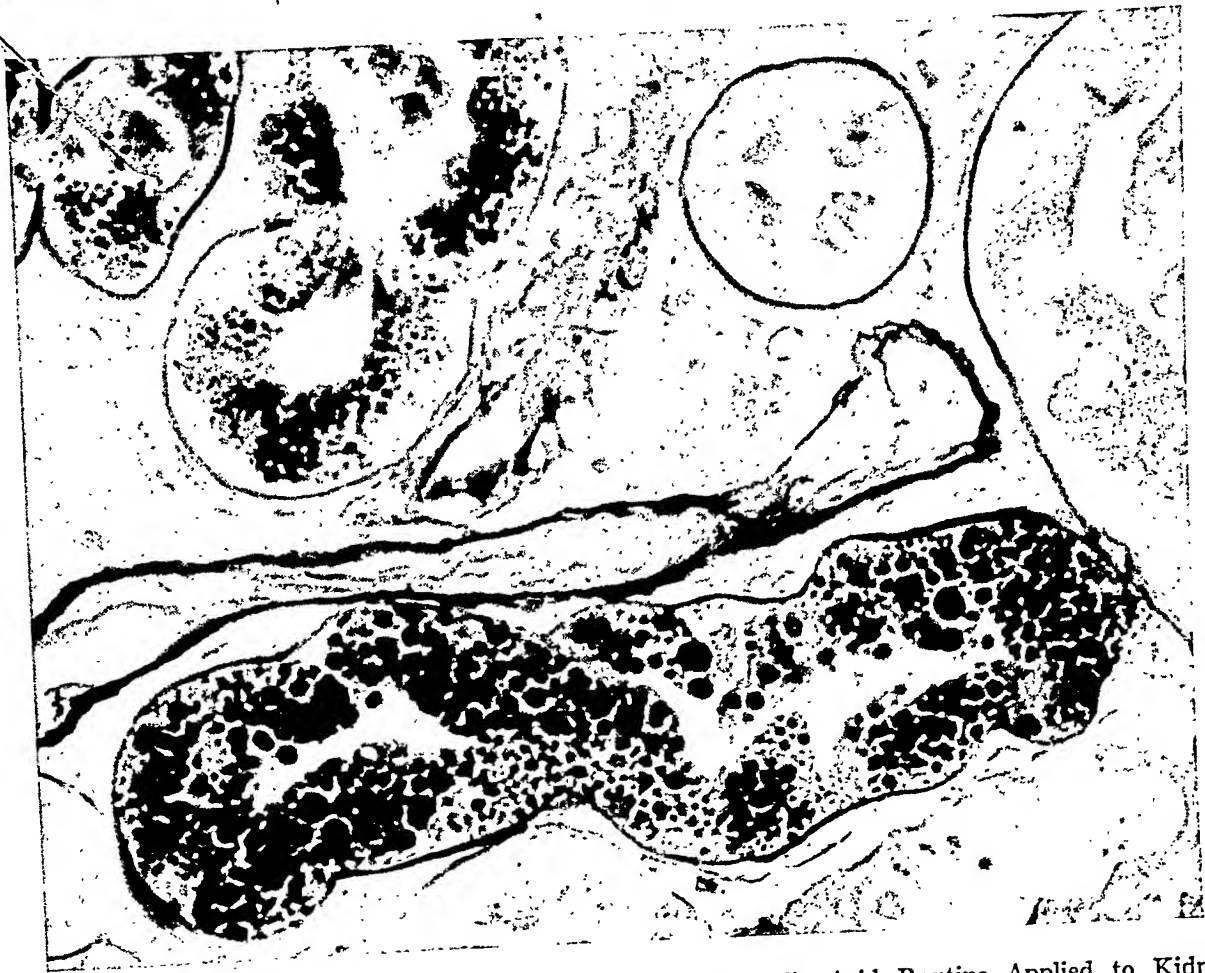
FIG. 5. Area from the generally normal kidney of a white female, 46 years old, dying with metabolic craniopathy. An obsolescent glomerulus is seen. There are several segments of a tubule in process of disappearance and showing thickening of the basement membranes. The arteriole shows several patches of hyalin. $\times 260$.

FIG. 6. From the same case as used for Figure 5. Several tubules, the cells of which contain hyaline droplets, are seen. One other tubule is in process of disappearance. It shows a greatly thickened basement membrane and a diminished or absent lumen. $\times 950$.

5



6



Periodic Acid Routine Applied to Kidney

EPITHELIUM-LIKE INCLUSIONS IN THE HEART *

S. M. RABSON, M.D., and L. J. THILL, M.D.

(From the Department of Pathology, St. Joseph Hospital, Fort Wayne, Ind.)

The pathologic anatomist subscribes to the view, common to all sciences, that the exceptional condition is worthy of study, since it frequently is of aid in understanding the more usual deviations from normality. The report, then, of a clinically insignificant, but anatomically interesting, cardiac anomaly justifies itself. As so often happens and as remarked by Davidsohn,¹ the lesion was accidentally discovered at necropsy, and only microscopically.

REPORT OF CASE

Clinical History

A white married woman, 29 years of age (no. 46-7801), entered the St. Joseph Hospital, October 21, 1946. She had difficulty in breathing, generalized pain in the chest and upper part of the abdomen, cough, and weakness. These symptoms had recurred frequently since their onset in 1940. When she was 14 years old (1931) she showed evidence of heart disease; there was no definite history of rheumatic fever. In the last 6 years of life she bore three living normal children, the last having been born 6 weeks before death.

During the 3 days in which she was hospitalized before her death, the temperature was always subnormal, and the respirations moderately rapid. Except for a brief transient rise, the pulse rate was never above 66, and terminally was recorded as 60. Treatment was ineffective. The symptoms became rapidly worse, and the patient expired on October 24, 1946.

Necropsy Findings

The body was examined (no. 46-A-083) 3½ hours after death. There was universal acute passive congestion, and fluid was found in the pleural and pericardial cavities, and in the abdomen. The enlarged heart (515 gm.) was dilated. Rheumatic stigmata included mitral valvular scarring with insufficiency, and bilateral atrial mural endocarditis and pulmonary arteritis.

In the original microscopic section of the posterior leaflet of the mitral valve and adjacent left atrium and ventricle an unusual feature was noted with the naked eye. In the angle between atrium and ventricle (Fig. 1), in the general plane of the valvular ring, there were round, solid and hollow, smaller and larger areas stained blue by hematoxylin. Closer examination of this, as well as of additional sections from the same block, failed to uncover any physical bridge between these structures and either the endocardium or epicardium. The inclu-

* Received for publication, June 30, 1947.

sions extended irregularly for a very short distance into the atrial myocardium, but did not do so on the ventricular side. All were sharply circumscribed by the connective tissues in which they lay.

The basic cell of the anomaly had a varied shape. In the solid structures it was generally polyhedral, with a cuboidal form assumed by those in the peripheral layer or layers. The hollow formations usually were lined by cuboidal cells; the lumina could be assumed to have been formed by dissolution of the cells in the interior.

Centrally placed in each cell was a round to slightly oval, single nucleus with distinct limiting membrane. The chromatin was finely granular and well dispersed. At least one obviously larger chromatin granule was found in every nucleus, and a few nuclei had single, well formed nucleoli. Nucleolar formation was accompanied by thickening of the nuclear membrane, and by coarsening and concentration of the chromatin on the membrane. A few mitotic figures were identified.

The cytoplasm was moderately eosinophilic, dense, and nongranular. No cytoplasmic inclusions were seen.

Hollowing of the cellular formations was accomplished by disintegration of the central portions. Some cells exhibited pyknosis and cytoplasmic hyalinization. Others, greatly swollen, with multiple vacuoles, usually exhibited a distinct, thick, cellular membrane before disintegration. A few cells crumbled without going through any of these processes.

The larger spaces (Fig. 2), many of them confluent, were well filled with cellular detritus and cells in varied stages of degeneration. A few neutrophilic and eosinophilic leukocytes and an isolated red cell completed the picture. In a few lumina conglutinated eosinophilic material was partly or completely calcified.

Adjacent to the ventricular epicardium (Fig. 3) the early stage of luminal formation lent the appearance of sebaceous glands (Fig. 3). The central cells were greatly enlarged and the cytoplasm vacuolated almost to the point of disappearance. The nuclear membranes stood out as thick eosinophilic rings, and the centrally placed single nuclei were slightly shrunken. No intercellular bridges were identified.

Another space was filled peripherally with several concentric rings of acellular hyaline material about a central mass of cloudy amphoteric substance, probably lightly impregnated with lime salts.

The stroma of the area was dense, generally collagenous, poorly cellular, and very vascular. The numerous vessels were chiefly capillaries and arterioles, but a few small arteries were seen. Lymphoid

cells and fewer plasma cells were congregated in appreciable numbers in the neighborhood of the large hollow formations.

DISCUSSION

The recent paper of Anderson and Dmytryk² thoroughly reviewed most of the available literature on primary cardiac tumors, as well as that dealing with epithelium-like inclusions, some similar to those described in this paper. In their case of a myxomatous tumor (the result of changes in thrombotic material) of the right atrium, gland-like and cystic structures were found in the new growth. These structures were lined by a variety of cells, ranging from flat cells of endothelial character to those of columnar type. Mucicarmine-positive material was identified in the latter, and cilia were suggested but not positively recognized. The inclusion of pericardial elements in the atrial wall during cardiac development was suggested as the causative factor.

Rezek³ was kind enough to furnish slides of the case he reported in 1938, and also to review material of the lesion here recorded. In his sections the epithelium-like character was, in general, not as pronounced as in the illustrations in his paper. He called attention⁴ to the sebaceous gland-like character of some structures in the present case, as described above, and inquired whether these were "predestined to develop into skin." Notwithstanding superficial appearances, we are reluctant to accept this suggestion, particularly in the absence of intercellular bridges.

The cellular spaces illustrated in the publication of Perry and Rogers⁵ closely resemble those of the case reported here. This was confirmed by Rezek,³ who had received slides from these authors after they had published their findings. He noted solid nests with cells like those of sebaceous glands or vacuolar degenerated epithelial cells. Perry and Rogers' diagnosis was "lymphangio-endothelioma," and histogenesis was ascribed to "lymphatic vasoformation." Rezek, in the text of his own original report, spoke of lymphangio-endothelioma. He added, however, that if the structures were epithelial, they might have resulted from the fetal inclusion of adjacent tissues, like the foregut (esophagus). The title of his paper showed the weight of his opinion by the inclusion of the phrase, "primary epithelial tumor."

Cornifying stratified squamous epithelium and sweat-gland-like apparatus were found by de Chàtel⁶ who referred to ectodermal heterotopia, or metaplasia of retained entodermal cells. Kolatschow,⁷ David-

sohn,¹ and Bayer⁸ each described cysts lined by ciliated columnar epithelium in the left ventricular papillary muscle. Kolatschow spoke of dystopia, as well as the possibility of an epicardial anlage with metaplasia to cylindrical epithelium. Davidsohn did not support any theory, and Bayer suggested a common origin with the bronchial wall.

We have no theories of our own. The propinquity, early in fetal life, of epithelium-producing tissues to the cardiac structures renders dystopia, heteropia, or inclusion⁸ especially attractive. In the absence of definite identification of the abnormal formations as epithelial, an endocardial, or epicardial origin seems more reasonable. The paucity of published observations on such solid and hollow inclusions makes the formulation of opinion additionally difficult. One suspects the meagerness is real, rather than relative, because the heart is more intensively and extensively studied at necropsy than any other organ.

SUMMARY

In the subepicardium and adjacent left atrial myocardium in the plane of the mitral ring of a white woman with rheumatic heart disease, abnormal structures were found. These were both solid and hollow, and had an epithelial character which could not be established beyond doubt. A cardiac origin, including epicardium and pericardium as possible sources, is probable, in consonance with similar, previously published reports.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

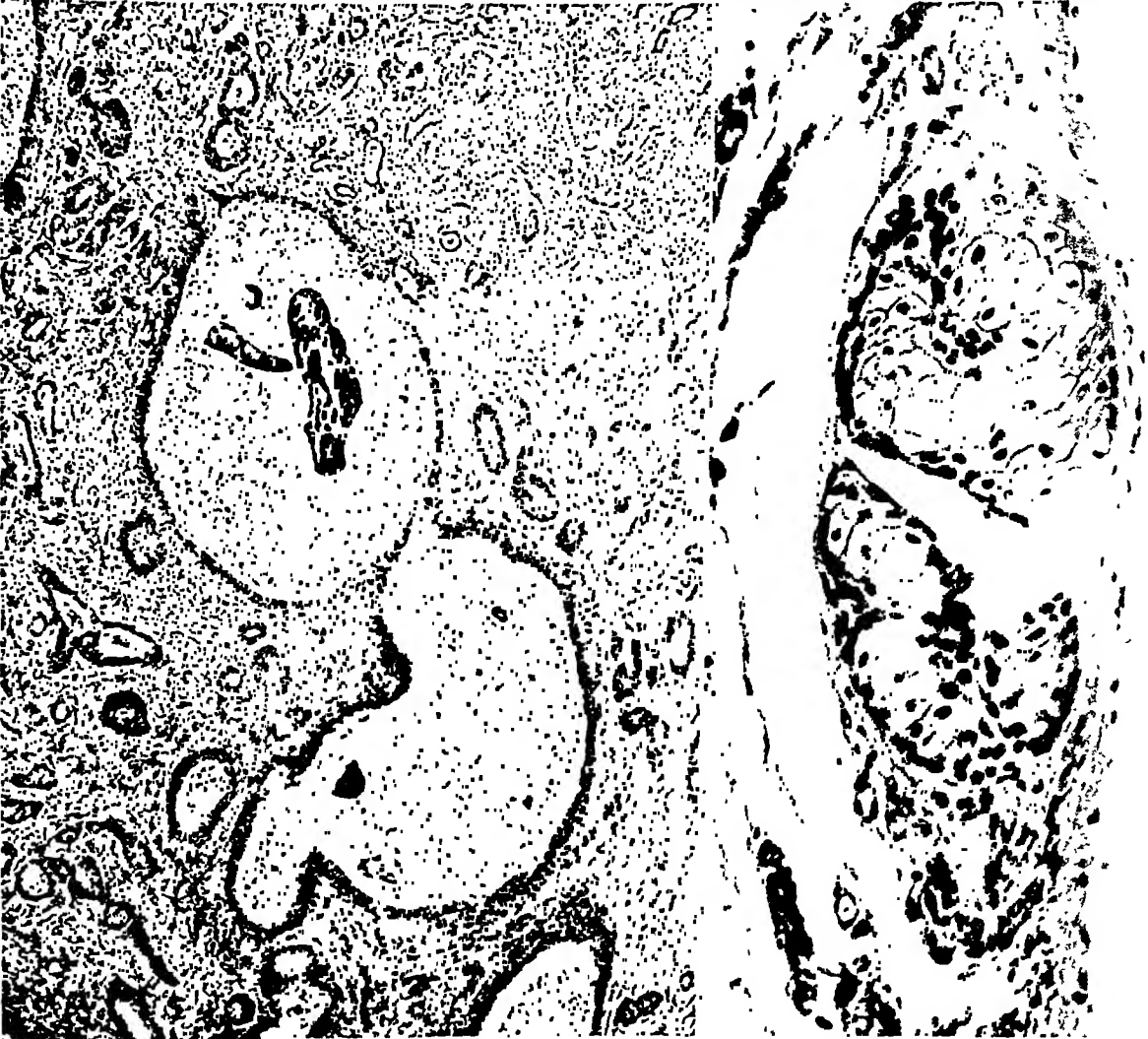
PLATE 119

- FIG. 1. Epicardium and subepicardium of the left atrioventricular angle, showing solid and cystic epithelium-like elements. Hematoxylin and eosin stain. $\times 32$.
- FIG. 2. Portion of the subepicardium showing cystic epithelium-like structures. Desquamated and broken-down lining cells form the contents of the cyst; calcification of intracystic necrotic material. Hematoxylin and eosin stain. $\times 50$.
- FIG. 3. Epicardial layer and subepicardial structure suggestive of sebaceous gland. Hematoxylin and eosin stain. $\times 215$.

1



2



FORTY-FIFTH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS
PHILADELPHIA
MARCH TWELFTH AND THIRTEENTH, 1948

THE AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

Forty-Fifth Annual Meeting,

Jefferson Medical College,

Philadelphia, Pennsylvania

March Twelfth and Thirteenth, 1948

PRESIDENT SOULE IN THE CHAIR

BUSINESS MEETING

March Twelfth, 1948

For the Council, the Secretary announced the following actions:

Election of new members

Carlton Auger, Quebec City, Que.	Thomas J. Moran, Danville, Va.
William H. Bauer, St. Louis	Gert L. Laqueur, San Francisco
Joseph L. Bernier, Washington, D.C.	Pablo Mori-Chavez, Lima, Peru
Maurice M. Black, Brooklyn	Joseph I. Mossberger, Denver
Charles Breedis, Philadelphia	Hans N. Naumann, Taunton, Mass.
Charles W. Buggs, Detroit	Manuel D. Peñas, St. Louis
Francis C. Coleman, Des Moines	Louis C. Posey, Birmingham, Ala.
John T. Cuttino, Durham	Dexter L. Reimann, Baltimore
Richard Ford, Boston	Russell A. Runnells, East Lansing, Mich.
Ira Gore, Washington, D.C.	Olaf K. Skinsnes, New York
John D. Hamilton, Kingston, Ont.	Reuben Straus, Beverly Hills, Calif.
Philip H. Hartz, Willemstad, Curaçao, N.W.I.	W. T. S. Thorp, Bethesda, Md.
Eugene Hildebrand, Great Falls, Mont.	J. P. Tollman, Omaha, Neb.
Arthur A. Humphrey, Battle Creek, Mich.	Richard C. Wadsworth, Bangor, Me.
Oscar B. Hunter, Jr., Washing- ton, D.C.	Prem N. Wahi, Agra, U.P., India
Aaron Kellner, New York	Frederic J. Wohlwill, Hathorne, Mass.
Benjamin H. Landing, Boston	Charles L. Yuile, Rochester, N.Y.
	Edwin E. Ziegler, Lancaster, Pa.

Reinstatement to membership of Drs. Harold M. Dixon and John W. Miller.

Acceptance, with regret, of the resignations of Drs. Warren C. Corwin, Gilbert Dalldorf, Harold W. Lyall, Peter K. Olitsky, and Ralph G. Stillman.

With deep regret, the deaths of Drs. Clement C. Fenton, Robert Green, Robert N. Nye, Cornelius A. Hospers, Francis P. Parker, and O. T. Schultz.

Upon nomination of the Council, the Association elected the following officers:

<i>President</i>	E. W. GOODPASTURE
<i>Vice-President</i>	SHIELDS WARREN
<i>Secretary</i>	HOWARD T. KARSNER
<i>Treasurer</i>	ALAN R. MORITZ
<i>Assistant Secretary</i>	HERBERT Z. LUND
<i>Assistant Treasurer</i>	SIDNEY FARBER
<i>Incoming Member of Council</i>	WILLIAM H. FELDMAN

The President announced that the American Type Culture Collection is an actively growing solvent concern housed in its own building in Washington. He said that at present there are not less than 5,000 cultures. Duplicate cultures are kept in a separate repository so that if those in the building are destroyed it will be possible to replace them. It was pointed out that the entire income is from sale of cultures. Dr. Soule suggested that members who receive requests for cultures refer these requests to the American Type Culture Collection, 2029 M Street, N.W., Washington, D.C., so far as possible.

The Secretary announced the re-election of Dr. Malcolm H. Soule as Assistant Editor of *The American Journal of Pathology* for the ensuing year, and the election of Dr. Tracy B. Mallory to the Editorial Board for a period of six years.

The Secretary announced the nomination of Dr. Malcolm H. Soule as representative of The American Association of Pathologists and Bacteriologists in the Division of Medical Sciences, National Research Council, for a term of three years beginning July 1, 1948.

For the Council, the Secretary announced that the next meeting of the Association will be held in Boston on April 15 and 16, 1949. The topic for the symposium for the 1949 meeting is "Pathology in Forensic Medicine." Dr. Alan R. Moritz has been appointed to act as referee.

REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted by the Council. It was accompanied by a certification from the Edwin S. Morse Company, Public Accountants, Boston. In condensed form, the Treasurer's report follows:

General Checking Account

Receipts

Balance on hand, January 1, 1947		\$ 2,740.64
Membership dues:		
Current	\$ 7,290.00	
Back	90.00	
Advance (1948)	30.00	
Interest on bonds, from investment account	590.00	
Sale of U.S. bonds, from investment account	6,044.04	
		<hr/>
		14,044.04
		<hr/>
		\$16,784.68

Disbursements

American Journal of Pathology (\$8.00 per member)		\$ 5,456.00
C. E. Lennon (Secretary to Dr. Karsner)	\$ 200.00	
P. A. Glass (Secretary to Dr. Moritz)	150.00	
Reporting 1947 meeting	213.08	
Attending meetings (officers)	192.32	
General office expense (secretary), including		
expense for annual meeting	212.37	
General office expense (treasurer)	44.30	
National Society Medical Research	50.00	
Auditing service	30.00	
Safe deposit box	6.00	
Bank charges	3.44	
Transfers to investment account	8,044.04	
		<hr/>
		9,145.55
		<hr/>
		\$14,601.55

Balance on hand, December 31, 1947 \$ 2,183.13

Investment Account

Balance, January 1, 1947 \$32,155.12

Receipts

Interest, savings bank accounts	\$ 114.08
Gain on U.S. bonds sold	44.04
Franklin Savings Bank	4,000.00
Provident Institution for Savings	4,000.00
	<hr/>

8,158.12

\$40,313.24

Disbursements

U.S. bonds sold (3% of 6/15/48)	\$ 6,000.00	6,000.00
Balance, December 31, 1947		<u>\$34,313.24</u>

Inventory of Investments

December 31, 1947

U.S. bonds 2½, series G	\$20,000.00	
Provident Institution for Savings	4,000.00	
Franklin Savings Bank	4,000.00	
Cambridge Savings Bank	4,216.17	
National Shawmut Bank	2,097.07	
Total		<u>\$34,313.24</u>

SCIENTIFIC PROCEEDINGS

A STUDY OF THE BEHAVIOR IN TISSUE CULTURE OF LYMPH NODES FROM HODGKIN'S DISEASE. Antonio Rottino, and (by invitation) Barney Worken and Augusta Hollender, New York, N.Y.

Abstract. Lymph nodes from 25 patients with Hodgkin's disease and 26 patients with non-Hodgkin's disease were studied in tissue culture.

In the Hodgkin's series, the following were conspicuous: liquefaction of medium, formation of multinuclear giant cells, phagocytosis, and occurrence of intracytoplasmic inclusions.

For the ensuing discussion we shall enlarge on the phenomenon of liquefaction. The conclusions drawn from this study are:

1. Liquefaction of medium in tissue culture is nonspecific.
2. It is more frequent and of greater degree in Hodgkin's than in non-Hodgkin's cultures.
3. Liquefaction occurs more often and to a greater degree with the granulomatous form of Hodgkin's disease and is diminished as the node becomes more fibrotic and sarcomatous.
4. Liquefaction probably represents digestive activity on the part of a fibrinolytic enzyme liberated from cells of one or more types. In Dr. Worken's opinion the ferment is elaborated by the reticular cell.

Discussion

(Dr. Herman Hoster, Columbus, Ohio) As I interpret Dr. Rottino's remarks concerning liquefaction, they appear to nullify, at least in part, the observations of Grand based on liquefaction. I should like to ask whether the inclusion bodies described by Grand are also considered nonspecific. I should like to inquire also whether there are any changes which Dr. Rottino considers specific in these tissue cultures.

(Dr. Jacob M. Ravid, New York, N.Y.) I should like to ask Dr. Rottino whether he has done any multiple tissue cultures on patients who have had several biopsies at various times during their illness, and if so, whether he observed in such cases any differences with regard to the liquefaction phenomenon.

(Dr. Rottino) With regard to the inclusion bodies, all sorts of inclusion bodies are seen in tissue cultures of lymph nodes from Hodgkin's disease. The same, however, are seen in control material. One source for cytoplasmic inclusions is the disintegrated remnants of cells dying in tissue culture and their subsequent phagocytosis by multinucleated giant cells and mononuclear cells. Present histochemical methods are not sufficiently refined to differentiate the histogenesis of all the inclusions that are found within cytoplasm. At present I think that most of the inclusions which we see arise by phagocytosis.

With regard to any specific phenomena occurring in Hodgkin's disease, I confess we have not seen any. The occurrence of Sternberg-Reed cells is mentioned; as far as I can make out, however, what have been identified as Sternberg-Reed cells are in my opinion multinucleated giant cells of the foreign body type that make their appearance in response to débris that occurs in tissue cultures.

In reply to Dr. Ravid's question concerning multiple biopsies and multiple cultures, we have observed cultures of different nodes removed at different times.

In 2 individuals the second culture showed the formation of numerous large multinucleated giant cells not present in the first cultures. In another instance in the first culture liquefaction was much more common than in the second culture.

HISTOPATHOLOGIC OBSERVATIONS IN CASES OF HODGKIN'S DISEASE TREATED WITH NITROGEN MUSTARD.* Virgil H. Cornell and (by invitation) A. S. Blauw, Washington, D.C.

Abstract. Several articles have reported temporary remissions, and a few relatively long periods of remission, in cases of Hodgkin's disease treated with nitrogen mustard. These have given no detailed descriptions of histopathologic changes subsequent to treatment, nor commented upon the absence of any changes. Other publications of animal experimental work during the war years, which have been recently released for publication, describe changes in great detail.

Seventeen cases, in which material before and after nitrogen mustard therapy was available, have been studied and no consistent histopathologic change can be found which may be attributed to the therapy. The present report is made to record the negative findings and warn against application of the animal tissue changes after sublethal dosage to the human cases treated by therapeutic dosage.

Discussion

(Dr. Antonio Rottino, New York, N.Y.) I had the privilege of doing an autopsy on a patient with Hodgkin's disease who died 3 days after a course of nitrogen mustard. The case was a late one. There were complications, such as a lobar pneumonia and severe anemia and leukopenia. At autopsy there was necrosis not only of the lymphocytes but also of the reticulum cells of all the lymph nodes we could find, as well as in the bone marrow.

UNUSUAL CHANGES IN LYMPHOSARCOMA UNDER NITROGEN MUSTARD THERAPY. Oscar B. Hunter, Jr. (by invitation), Washington, D.C.

Abstract. This paper deals with an apparent coagulation necrosis of lymphosarcoma tissue in a number of cases seen at the Army Institute of Pathology. These cases all show the same necrotizing effect of the tumor tissue without apparent effect on the relatively normal tissue. This effect has not been noted in the literature to date and in the instances detailed in the paper the tumor has apparently been eradicated almost completely.

Discussion

(Dr. V. H. Cornell, Washington, D.C.) I would like to say that the apparent discrepancy in the two papers which have just been presented may be reconciled. We have seen this change in autopsied cases given nitrogen mustard therapy; however, I think I have seen it also in cases that have never met nitrogen mustard. It was with this idea in mind that for over a year and a half I have been going back over our material and every time I saw something in cases that were treated with nitrogen mustard, I saw it also in cases that were not treated. I think that unless you look at this new treatment with great discrimination you cannot be sure that the criteria you are applying are specifically related to the therapy. I think there must be some action, and apparently some late action, of nitrogen mustard. It is very well described in animals with large

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

doses. As I stated, the effect ceases, perhaps, in 15 to 30 minutes within the body. Clinically the improvement is prompt but anatomically delayed. What I was afraid of was that the papers, which have been in preparation during the last year and are now coming out in the literature, on animal therapy with huge doses of nitrogen mustard might be interpreted as indicating what was occurring in the patient who receives the therapeutic dose. I simply urge everyone to be extremely strict in the interpretation of what they see.

(Dr. Shields Warren, Boston, Mass.) May I ask Dr. Cornell if he means to imply that the type of phagocytosis he showed is specific for the action of nitrogen mustard?

(Dr. Cornell) No, I thought I made that clear; the first group of slides showed phagocytosis and were nitrogen mustard cases, while the last three slides were from cases that had not had nitrogen mustard. I am simply trying to show the similarity of phenomena that were observed first in the nitrogen mustard cases, and could then be found in other cases.

(Dr. Hunter) I am glad we had a second biopsy in the first case, showing the histologic change before and after. It was very closely controlled.

ATYPICAL AMYLOID DISEASE, WITH OBSERVATIONS ON A NEW SILVER STAIN FOR AMYLOID.* Lester S. King, Chicago, Ill.

Abstract. From the group of so-called "primary" amyloidosis, a special subgroup of "atypical amyloidosis associated with senility" is distinguished. Five cases, all concerning patients over 80 years of age, are described, in which amyloid occurred in the heart, almost exclusively, and which showed no common denominator apart from advanced age. These are contrasted with a further case of "atypical amyloidosis associated with pyelonephritis." A new method of staining amyloid with ammoniacal silver is described.

Discussion

(Dr. Alfred Plaut, New York, N.Y.) How were the amyloid reactions with the customary methods? I ask the question because of the well known staining irregularities of atypical amyloid.

(Dr. Jesse E. Edwards, Rochester, Minn.) I should like to subscribe to the skepticism concerning the infrequency of amyloidosis of the heart in the comments of Dr. King. I think the frequency of this condition has not been appreciated in the past. In the last year we have seen 4 instances of the condition essentially similar to the 5 he showed. In each of these 4 the diagnosis was made on gross examination and the gross diagnosis was made on the basis of lesions seen in the endocardium, especially of the left atrium. The lesions were also seen in the right atrium. They appeared as "tapioca" lesions. The little specks may be numerous; they were innumerable in the endocardium of the left atrium and were smaller than those which we ordinarily associate with the tapioca lesions of amyloidosis of the spleen; they tended to be of pinpoint size, rarely a millimeter in diameter. Occasionally some of these lesions would involve the valvular endocardium. I wonder if Dr. King found, in his cases, lesions involving the endocardium. In the 4 cases I mentioned there were also lesions of amyloid in the myocardium.

Just a word about terminology: I think the term he recommends is a little vague. I do not know that I can suggest a better one, but I would suggest that in some way the terminology should definitely include amyloidosis with reference to the heart which the term "atypical amyloid disease" does not do.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

(Dr. Ralph D. Lillie, Bethesda, Md.) May I ask Dr. King if his silver reaction is based on formaldehyde-fixed material, or are other fixatives used?

(Dr. Friederich Wohlwill, Danvers, Mass.) I would like to ask whether, by chance, the nerves have been examined. I saw a case of atypical amyloidosis with the clinical picture of polyneuritis in which the main seat of the amyloid substance was in the spinal roots, the spinal root ganglia, and the peripheral nerves. But there was amyloidosis of the heart, too.

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder whether Dr. King will comment on the relationship of this substance to ordinary hyalin, realizing that hyalin is a rather all-inclusive term and that amyloid itself includes quite a complicated heterogeneous group of chemical substances. I ask the question because it is my impression that this is a rather common phenomenon in hearts as I see them. Your statistics do not represent the ordinary experience of the average pathologist, at least, judging from my experience. My interpretation of the lesion is that it represents a form of change in arteriosclerosis. The lesions in the glomeruli are very similar to what we see in the Kimmelstiel-Wilson lesion of the glomeruli and in hyalinosis of arterioles, and there we are fairly well agreed that each is a variety of arteriosclerosis. The noted localization in the vessels seems to bear out that impression. I would be appreciative if Dr. King would resolve this difficulty.

(Dr. Arthur C. Allen, New York, N.Y.) In regard to Dr. Angrist's comment, I should like to re-emphasize that lesions in diabetic glomerulosclerosis which have a similarity in routine sections to the glomeruli of amyloid disease do indeed have a striking argyrophilia, but that the argyrophilic material has a specific laminated pattern, which I believe is different from the homogeneous smudgy appearance of the argyrophilia of the amyloidotic glomeruli described here.

(Dr. King) In reply to Dr. Plaut's question, I should have emphasized that in all these cases the amyloid stains very well with methyl violet and Congo red, so that the staining reactions are entirely typical.

Before replying to Dr. Edwards, may I ask him the age of the patients in whom he found the disease?

(Dr. Edwards) I do not recall the exact ages, but I believe they were all older individuals.

(Dr. King) In the matter of terminology, the best I could think of was atypical amyloidosis associated with senility, and I used that because I do not believe the amyloid is necessarily restricted to the heart. There are observations in the literature describing amyloid in the seminal vesicles. Unfortunately in these cases the seminal vesicles were not examined histologically, but in a series of reported cases in which the ages were over 80, almost 70 per cent showed amyloid in the seminal vesicles. My feeling is that amyloid deposition in the heart and in the seminal vesicles is a function of age. I have observed it in the heart. I may say that we had not known of any in the peripheral nerves.

In reply to Dr. Lillie's question, the material is formalin-fixed. The only striking feature of this impregnation is that no reduction is necessary.

In regard to Dr. Angrist's question, he is asking me a question which the pathology students are asking, and usually we find that the questions which the pathology students ask are the most difficult to answer. The only way I can tell the difference between amyloid and other hyaline substances is by specific stains. My feeling is that any hyaline material which reacts positively with the accepted amyloid stains—Congo red, methyl violet, iodine, and, I hope, this technic—is amyloid, and if it does not react with these methods it should not

be called amyloid. I agree with Dr. Angrist that this is probably a common condition. However, the importance of applying specific amyloid stains to this condition has not been appreciated. In my experience we have had 20 cases over the age of 80, and in these 4 showed this condition. I have not tried the technic on any examples of intracapillary glomerulosclerosis.

ENHANCEMENT OF METASTASIS OF A MOUSE MAMMARY CARCINOMA FOLLOWING ROENTGEN IRRADIATION. Edwin D. Murphy and Henry S. Kaplan (by invitation), Memphis, Tenn.

Abstract. The tumor studied was Dr. Halsey Bagg's tumor no. 755 which grows slowly in C57 black mice, seldom metastasizing. In 4 experiments, groups of 40 to 50 C57 mice were inoculated, and when the tumor reached a diameter of 1 cm., half of the mice received doses of 400 to 1000 r. applied locally to the tumor, the rest of the mice being held as controls. The incidence of pulmonary metastasis after 6 weeks was 44 per cent in the case of the irradiated tumors and 10 per cent in the controls. Heterotransplantation studies carried out collaterally indicated that progressive growth was more readily obtained with irradiated tumor than with untreated tumor, suggesting a primary effect upon the tumor rather than directly upon the host.

Discussion

(Dr. Alfred Plaut, New York, N.Y.) Was this the original strain from the Jackson Memorial Laboratory, or a sub-strain?

(Dr. Murphy) The strain was Strong's sub-line of the C57 black, but, as I indicated, we carried the tumor in that strain for a period of 2 years before this experiment was started. We have carried on some observations on metastasis for another type of work, and are using that as our background for the behavior of the tumor. The incidence of spontaneous mammary carcinoma is low and none appeared in any of our animals in the age group used.

COLOR AND PRECIPITATION REACTIONS WITH MALIGNANT TUMORS. Emil Weiss, Chicago, Ill.

Abstract. Fresh unfixed tissue of malignant tumors (0.25 to 0.5 cc. suffices) is cut to small particles, placed in a tube, covered with 10 cc. of a saturated solution of litmus in 70 per cent acetone, corked and shaken vigorously for 1 minute. Malignant tissue turns the solution red, while the control containing normal or benign tissue remains violet. A solution of 20 per cent salicylic acid in acetone, used in the same way, gives precipitation with malignant tumors and none with benign or normal tissues. Controls for each reaction are: (1) Reagent control containing the reagent used in the test; (2) positive control containing the reagent and a known malignant tissue; (3) negative control containing the reagent and a known normal tissue. The controls are handled in the same way as the unknown. The test is interpreted as strongly positive if salicylic acid and litmus give the described reactions for malignant tumors, as weakly positive if only salicylic acid gives the typical reaction, and as negative if precipitation does not occur with salicylic acid regardless of the litmus reaction. Impurities of proteins in the test tubes may cause false positive reactions and acids may cause false negative reactions. The test gave results corresponding with histologic examinations in 95.17 per cent of malignant tumors (80.12 per cent strongly positive, 15.06 per cent weakly positive reactions). Benign tumors gave

4.24 per cent positive reactions (only weakly positive). The test applies to all types of malignant tumors.

Discussion

(Dr. Russell L. Holman, New Orleans, La.) I would like to ask Dr. Weiss if he has tried this reaction on serum, or urine, or on extracts of normal tissue.

(Dr. Weiss) This reaction cannot be used on serum because it would coagulate the serum. For urine tests the reagents would have to be used in a more concentrated form and adjusted accordingly. Extracts of 200 normal tissues gave satisfactory results.

GRANULAR CELL "MYOBLASTOMAS" AND GRANULAR CELL NEUROFIBROMAS: SEPARATION OF NEUROGENOUS TUMORS FROM THE MYOBLASTOMA GROUP. John A. Fust (by invitation) and R. Philip Custer, Philadelphia, Pa.

Abstract. Study of 51 tumors originally diagnosed granular cell myoblastoma at the Army Institute of Pathology and the Presbyterian Hospital disclosed certain structural differences between all but one occurring in the tongue and those located elsewhere. Fifteen of the 16 lingual tumors, if they are true tumors in the neoplastic sense, appeared to arise through alteration of pre-existing voluntary muscle fibers. The 16th was a polypoid tumor bearing no demonstrable relation to muscle and resembling the granular cell tumors found in the skin and subcutaneous tissue at a variety of other sites. In the 35 non-lingual cases we believed it possible to demonstrate a histogenetic relationship to peripheral nerves, perhaps to Schwann cells, which are known to have granular cytoplasm under certain conditions. Large granular cells were frequently found within the perineurium of nerve twigs well beyond the reaches of the tumors, appearing to have developed there rather than having invaded the nerves. Four other tumors showed gradations between the conventional type of neurofibroma and granular cell tumors.

In summarizing this series of 51 cases of granular cell tumors:

1. Those that appear to arise in muscle occurred only in the tongue. Whether they are true neoplasms is not yet clear.
2. Those that appear to arise from nerves may occur in the tongue, but are found mostly elsewhere in skin and subcutaneous tissue. They are true neoplasms.
3. These 2 groups are structurally different, having in common the granular cytoplasm of their component cells.

Two other types of granular cell tumors, not included in this series, may prove to be separate entities. The first is the so-called congenital epulis. The second is found in skeletal muscle, notably in the thighs and buttocks; it differs somewhat in histologic pattern from the lingual lesions and may be more closely related to the conventional rhabdomyoma.

Discussion

(Dr. Jacob M. Ravid, New York, N.Y.) Did any of these granular cell myoblastomas show evidence of malignancy?

(Dr. Fust) All of these tumors appeared to have been cured by simple removal. The only indication of malignancy was the occasional occurrence of carcinoma *in situ* over the tumor. There was one case in the lingual group in which Dr. Custer thought that carcinoma *in situ* was a reasonable diagnosis, and there were 4 among the neurogenous tumors.

HIBERNOMA. REPORT OF CASE. Osborne A. Brines and (by invitation) M. Harvey Johnson, Detroit, Mich.

Abstract. For many years the existence of brown multilobular fat in hibernating mammals and some nonhibernating rodents has been recognized. These masses which are of considerable size have been given a variety of names, the best known of which are hibernating gland and interscapular gland. The hibernating gland is described as being yellowish brown, lobulated, somewhat resembling pancreas or salivary gland, and composed of coarsely granular or finely multilobular cells containing considerable protein but with a smaller fat content than the cells of ordinary fat. Since 1905 approximately 6 neoplasms derived from this structure have been reported in the medical literature. In 1915 Gery reported a case and proposed the name hibernoma, which has been accepted by later contributors. The literature also contains reference to several additional cases which seem to be reasonably authentic but which could be accepted only with reservation. Confusion exists over some "atypical lipomas." Attention is called to the similarity between this tumor and granular cell myoblastoma. It is reasonably possible that some of the former have been mistaken for the latter. Such possible confusion could be eliminated by the employment of fat stains in all granular cell soft tissue tumors. A case of a hibernoma occurring in the right scapular region in an 18-year-old female is reported. The specimen was a nonencapsulated, somewhat flattened tumor measuring 10 by 4 by 2 cm., which was grayish brown and composed of longitudinal parallel bundles having the general appearance of skeletal muscle. There was no recurrence at the end of 1 year.

Discussion

(Dr. Frank Dutra, Cincinnati, Ohio) Is there any relationship between these apparently benign tumors and the malignant liposarcomas? My comment is based on the fact that in benign lipomas foam cells are less common than in those with malignant potentialities.

(Dr. Brines) It is true that foam cells are found in some lipomas. As far as I know, no hibernomas have been reported as malignant or have pursued a malignant clinical course. This patient I have observed only a short time and she has shown no evidence of recurrence. I think it is important, however, that these tumors be differentiated from the so-called atypical lipomas or possibly low-grade liposarcomas, and particularly from granular cell myoblastomas—a mistake which can be avoided very easily.

SCLEROSING LIPOGRANULOMA. Hans F. Smetana and William G. Bernhard, Washington, D.C.

Abstract. The 8 cases presented were characterized by a peculiar tumor-like swelling, persisting from 5 weeks to 12 years, without systemic reaction. This condition occurred in males between the ages of 25 and 43 years and involved the scrotum, spermatic cord, or penis in 6 instances and the buttocks in 2. A history of trauma was recorded in 2 cases, while an inflammatory process of the penis preceded the appearance of the swelling in 2 others. No history of trauma was elicited in the remaining 4 cases.

The clinical picture was that of progressive noninflammatory swelling compatible with neoplasm. In 7 cases a cure was effected by surgical excision, but in one instance the swelling recurred several times despite surgical intervention. The "tumors" removed at operation varied from 3 to 8 cm. in diameter and con-

sisted of fatty tissue, with partial substitution by fibrous bands containing cystic spaces.

Histologically, there was replacement of the subcutaneous fat tissue by fat globules of unequal size, separated by fibrous septa which were infiltrated by mononuclear wandering cells and a few eosinophils and polymorphonuclear leukocytes. Many of these fat globules were surrounded by multinucleated foreign body giant cells. The predominant cells were macrophages which had phagocytized fat droplets; small free fat droplets were present also in the fibrous septa and in tissue spaces. Some of the vessels showed perivascular, predominantly lymphocytic, infiltrations. Fat droplets, free as well as surrounded by foreign body giant cells, were seen in perivascular and perineural lymphatics. Occasionally the parenchyma of regional lymph nodes was partly replaced by fat in macrophages, lining cells of sinuses, and foreign body giant cells. Fibrous tissue showing a tendency to hyalinization was present between the fat globules of the subcutis, roughly corresponding in amount to the age of the lesion; in chronic cases there was extreme hyaline scarring with foci of calcification.

Histogenetically, the earliest lesion discernible was focal necrosis of some of the septa of the subcutaneous fat tissue with subsequent confluence of fat droplets to large, irregularly shaped globules. Inflammatory cells appeared and many of the free fat droplets were taken up by macrophages; some of these then migrated into the lymphatics and tissue spaces. Macrophages became so greatly distended by the engulfed fat droplets that their cytoplasm gradually disintegrated, again setting free the fat. By that time, foreign body cells had formed surrounding the fat globules. Neither fatty acid crystals nor soap formation were observed. The size of the globules varied, the larger ones being small spaces, surrounded by numerous multinucleated giant cells. Fibroblasts and connective tissue elements became more and more evident, finally incarcerating the fat globules in scar tissue.

The pathogenesis of this process is not clear. It appears, however, that certain injuries of the subcutaneous fat tissue may cause a profound local disturbance of fat metabolism in some individuals, which leads to liberation of fatty substances acting as foreign bodies, with consequent foreign body reaction. This reaction is similar to that observed in the lungs in cases of lipoid pneumonia and in the pelvic tissues after uterosalpingography with lipiodol. There is also great similarity to the local histologic processes observed in Weber-Christian disease and traumatic fat necrosis of the breast.

Discussion

(Dr. Lester S. King, Chicago, Ill.) I should like to ask what Dr. Smetana considers the relation of this to Weber-Christian disease.

(Dr. Wiley D. Forbus, Durham, N.C.) Dr. Smetana's problem is very interesting to me, especially, because of the possible influence that fat may have over the motility of the reticulo-endothelial cell. We have been working recently on the effects of peanut oil in the lung, and we have found, quite contrary to what Dr. Smetana has seen, that the peanut oil, although phagocytosed, remains in the lung and is not transported to the lymph nodes. I wonder if the fats which were present in Dr. Smetana's materials are more effective in stimulating the movement of phagocytic cells than other forms of fat. In Whipple's disease, about which you will hear in the next paper, the fat appears in a very strange form in macrophages in the intestinal mucosa. That fat stimulates the movement of the macrophages to an extraordinary degree, and you will find in the lymph nodes of the mesentery great quantities of these fat-laden cells, but

the fat now has assumed a new form which provokes a marked reaction exactly like that which Dr. Smetana has shown. I wonder if Dr. Smetana will comment on the stimulative properties of different types of fat.

(Dr. Smetana) I consider the local histologic changes seen in Weber-Christian disease practically identical with those present in sclerosing lipogranuloma. The disease is, of course, differentiated from sclerosing lipogranuloma by its clinical features. In addition, while the lesions in Weber-Christian disease will disappear after some time with local atrophy of the involved fat tissue, we have not seen this course in lipogranuloma.

As to the influence of different fats on the activity of macrophages, I believe the paper by Henry Pinkerton (The reaction to oils and fats in the lung, *Arch. Path.*, 1928, 5, 380-401) deals with this subject. He described the deposition of animal and mineral oils in bronchial lymph nodes after intratracheal instillations, while vegetable oils were not transported to the lymph nodes. This may be related to the absence of specific lipases for mineral and vegetable oils in the animal body. The transportation of fat to the regional lymph nodes in cases of human lipogranuloma is therefore the more remarkable since this phenomenon indicates absence of splitting of autogenous fat. It may be suggested that a subtle chemical change of the subcutaneous fat occurs during its extrusion from the normal fat cells which renders it resistant to splitting; however, in the absence of chemical studies, this can only be assumed.

LIPODYSTROPHY INTESTINALIS (WHIPPLE'S DISEASE). B. Black-Schaffer, and (by invitation) J. P. Hendrix and P. Handler, Durham, N.C.

Abstract. The pathologic anatomy and physiology of lipodystrophy intestinalis (Whipple's disease) is re-examined in the light of 4 new cases. The disease may be recognized by nonlipid macrophagocytosis of the lamina propria of the small intestine and occasionally the proximal colon, lipogranulomatosis of the mesenteric and draining lymph nodes, and the absence of significant evidence of chylous obstruction. Because of the uniform absence of chylous obstruction, the presence of steatorrhea, and poor glucose absorption (glucose tolerance tests) it is postulated that, as in sprue, lipodystrophy intestinalis is produced by a functional defect of the enteric epithelium resulting in decreased ability to absorb fats as well as glucose and possibly proteins.

Lipodystrophy intestinalis differs from sprue in that it is marked by pathognomonic anatomic changes and does not result in the development of macrocytic anemia.

The nature of the nonsudanophilic material in the intestinal lamina propria and the lymph nodes is unknown. As preserved (Kaiserling's solutions), it cannot be identified chemically as lipid.

It is suggested that because of the high incidence of fibrous pericarditis and arthritis that Whipple's disease may be the sequel to an as yet unidentified systemic illness characterized by inflammation of the serous membranes.

Discussion

(Dr. V. H. Cornell, Washington, D.C.) We have had a case very similar to his which I should like to add to Dr. Black-Schaffer's series. I will call attention to two features which he has already demonstrated: one is the nonlipid macrophagocytosis of the intestine by the reticulo-endothelial cells and the other is the tumor-like growth in the lymph nodes. The authors, like myself, were unable to demonstrate the lipids which we expect in Whipple's disease, but we

arrived at a slightly different conclusion, in that they conceived it as an inherent inability of the reticulo-endothelial system to take care of the fat that is brought to it, and therefore the disease is really a reticulo-endotheliosis, in which the cells are biologically deficient.

(Dr. Arthur C. Allen, New York, N.Y.) With regard to Dr. Black-Schaffer's theory of disturbed glucose metabolism as a factor in Whipple's disease, I should like to ask if he has had the opportunity to do histologic phosphatase studies in these cases.

(Dr. Black-Schaffer) We have not done any phosphatase studies in these cases. We should like to point out that phosphorylation is not always an essential step in absorption of carbohydrates. Fourman, utilizing xylose, has published this fact in a recent issue of *Clinical Science*. I would hesitate to accept Whipple's disease as an example of a primary reticulo-endotheliosis. We believe it to be an example of nonlipid and, in the lymph nodes, lipid phagocytosis, secondary to a functional defect of the enteric epithelium. There does not appear to be any disturbance of the reticulo-endothelial cells.

FACTORS INFLUENCING THE PATHOGENESIS OF EXPERIMENTAL OVARIAN TUMORS IN RATS. G. R. Biskind, and (by invitation) R. Pencharz and M. S. Biskind, San Francisco, Calif., and New York, N.Y.

Abstract. The hormonal imbalance that follows transplantation of an ovary into the spleen of a castrate rat initiates the development of a luteoma through stages of continuous formation and enlargement of corpora lutea. After a prolonged period a granulosa cell tumor appears in the luteoma. These tumors do not appear if the rat is hypophysectomized. In rats that retain one normal ovary and that have the other ovary transplanted to the spleen, the transplanted ovary atrophies and the normal ovary hypertrophies. If the normal ovary is removed after the ovary in the spleen has undergone atrophy, the latter resumes growth and progresses through the stages of continuous luteal formation and enlargement as noted in the original tumors.

Discussion

(Dr. Howard T. Karsner, Cleveland, Ohio) Naturally I am deeply interested in this excellent study. Has time permitted determination of whether these tumors are transplantable?

(Dr. Russell L. Holman, New Orleans, La.) Were any feminizing characteristics associated with these tumors, or couldn't you tell about that?

(Dr. Lester S. King, Chicago, Ill.) Were any vaginal smears made?

(Dr. Antonio Rottino, New York, N.Y.) Is it possible to transplant the ovary of one animal into the spleen of another animal?

(Dr. Biskind) In answer to Dr. Karsner's question, we have some studies on transplantability, but they are not complete. It is extremely difficult to transplant the tumors that develop in rats; however, the tumors that develop in mice are readily transplantable. I have not completed our experiments on anterior chamber transplantations.

The feminizing effect occurred in some granulosa cell tumors. These tumors elaborated so much estrogen that it passed through the liver and produced cornification of the vagina and uterus.

Vaginal smears are performed routinely. In the first period after transplantation the predominant cells were leukocytes, occasionally epithelial cells were

evident. Later, as the granulosa cell tumor is developing, a smear composed of cornified epithelial cells may replace the castrate smear.

The transplantation of the ovary of one animal to the spleen of a castrate rat, male or female, is readily accomplished and all transplants "take."

VIRILIZING HILUS CELL TUMORS OF THE HUMAN OVARY WITH A REVIEW OF OVARIAN HILUS CELLS AND EVIDENCE OF THEIR ANDROGENIC FUNCTION.

William H. Sternberg (by invitation), New Orleans, La.

Abstract. The hilus of the adult human ovary normally contains nests of cells morphologically identical with Leydig cells and whose function is probably androgenic. The morphology of these cells (originally described in detail by Berger) is discussed and their relationship to nonmyelinated nerve and vascular spaces emphasized. Particular stress is placed upon the finding in the cytoplasm of crystalloids of Reinke, structures considered specific for Leydig cells. Two instances of tumors of these cells with masculinizing syndromes are presented. One similar well established case exists in the literature, but probably others have been misdiagnosed. Two additional cases of giant ovaries with stromal hyperplasia and clinical masculinization, in which the hilus cells were increased in number, are presented. There is also evidence that these cells respond to chorionic gonadotrope.

Discussion

(Dr. Jacob M. Ravid, New York, N.Y.) Was any biochemical work done on these tumors, and what specifically is the difference between them and those of the clear cell group which are also virilizing in nature, and for which Rottino and McGrath created the term of "masculinovoblastoma"?

(Dr. A. R. Kantrowitz, Brooklyn, N.Y.) Were any polarizing microscope studies performed on frozen sections of the tumors and normal control ovaries?

(Dr. Sternberg) In answer to Dr. Ravid's first question, not many histochemical studies have been done on this curious group of cells. The Feulgen-Verne reaction is positive as it is in the Leydig cells of the testis. I am not aware of any additional studies of a chemical nature that have been done on these cells. Additional studies are being done.

We feel that this group of virilizing hilus cell tumors is different from the so-called group of masculinovoblastomas. The hilus cell tumors derive from specific cells present in the normal ovarian hilus, which have all the morphologic characteristics of Leydig cells, including the unique crystalloids of Reinke. This is, then, a specific tumor derived from a single cell type normally present in the ovary. I have no doubt that some instances of masculinizing tumors, classified in the literature as masculinovoblastomas, adrenal rest tumors, and luteomas, may have been misdiagnosed and are in reality tumors of this type. I believe that there is such a thing as an adrenal rest tumor but that it is morphologically distinct from hilus cell tumors.

PIGMENTED NEVUS: FACTORS OF AGE AND ANATOMICAL SITE. Herbert Z. Lund and (by invitation) G. Dorr Stobbe, Cleveland, Ohio.

Abstract. To establish a basis of comparison in the study of melanoblastomas, 200 pigmented nevi, selected from four general cutaneous sites, were divided according to age intervals. Lentigo and blue nevi were excluded. Of the features studied only a few can be presented.

Although origin from and relationship to follicles and sweat glands, besides the epidermis, influenced shape, depth, and pigmentation of nevi, it was found that nevi from all sites followed the same general pattern of development. In early life proliferation of cells in the epidermis, dermo-epidermal junction, follicles, and sweat glands is seen and, as a trend, there was diminution in later years both in degree and in percentage of nevi showing it. In successive decades of life the percentages of nevi showing moderate to marked proliferation were: 90, 44, 19, 19, 6, 7, and 0. The last figure represents all cases above 60. There was no abrupt cessation at a given age. The figures indicate either persistence of junctional proliferation from birth or incipience in any of the age intervals. Other histologic features are helpful in the distinction.

Traub and Keil doubt that junctional proliferation is part of the developmental process of benign nevi and give it the special significance of indicating a process which is "potentially malignant (precancerous) whether it arises early or late in life." The above data contradict this. It is part of the developmental process of benign nevi. Although melanoblastoma may have origin in a similar site, such a diagnosis requires other considerations and evidence.

The percentages of nevi in successive decades of life showing any mitotic figures at all were: 20, 12.5, 6, 0, 3, 0, and 0. The last figure represents all cases above 60. Mitosis, always uncommon, was found more often in childhood and youth, and occurred in the junctional as well as in the deeper cells.

As the junctional cells proliferate they migrate from the site of origin and differentiate to the usual "nevus cells." The accumulation of nevus cells separates epidermis from corium. Often proliferation about follicles and sweat glands occurs deeply in the corium. The cells become more fusiform and fibrillar with age. The percentages of nevi in successive decades of life showing much fibrillar proliferation were: 10, 25, 45, 58, 71, 79, and 100. The last figure represents all cases above 60.

Parallel with the increase of fibrils is the appearance of nerve-like elements. The percentages of nevi in successive decades of life showing such were: 0, 0, 13, 14, 26, 48, and 56. The last figure represents all cases above 60. Thus, the "neuro-nevi" are appearances in later stages of development.

It is seen that many of the so-called "types" of nevi are actually nevi in various phases of development. From a consideration of the features discussed, nevi can be classified as young, intermediate, or old.

Although there were variations in certain features of nevi according to anatomical site, this cannot be discussed in this limited presentation.

Discussion

(Dr. Arthur C. Allen, New York, N.Y.) I should like very strongly to endorse Dr. Lund's plea for a re-examination of the whole field of nevi and melanomas. On the other hand, in anticipation of work just completed by Dr. Spitz as well as by myself, and in view of the seriousness of the problem, it might be worth recording some disparity regarding one or two of the basic features which Dr. Lund postulated. It is beginning to be a fairly well known fact that metastasizing melanomas in children are extraordinarily rare; they occur, but they are rare. Nevertheless, as indicated by Dr. Spitz,* the histologic picture of the benign juvenile melanoma may be indistinguishable from those melanomas that kill after puberty. We feel most positively that the so-called junctional change after puberty acquires a peculiar and different significance and virulence that it does not have prior to puberty, and if disregarded, may lead to tragic developments. It is just this kind of junctional change that occurs almost constantly in

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the pigmented lesions of the soles of the feet in adults—a common site of melanomas notwithstanding the virtual absence of the ordinary intradermal nevus in this location.

(Dr. Lund) This presentation is, of course, over-simplified. I could not go into some of the problems which Dr. Allen raised. I will stress, however, that the finding of junctional proliferation alone does not label a nevus as being malignant. We have been disturbed by what appear to be young nevi—in some instances consisting of only junctional and intra-epidermal cells with practically no further differentiation—occurring in persons 20, 30, and 40 years of age. This problem is the subject of further analysis and is one of the reasons we undertook this investigation. Perhaps Dr. Spitz does have the answer and we anticipate the forthcoming publication. The distinction of benign and malignant may be equivocal, but so far in the series of melanoblastomas with metastasis that we are collecting, there have been deviations in the primary lesions from the pattern of development I have described for benign nevi.

The factors of anatomical location could not be given for want of time. The general scheme of development of nevi was similar, however. Neuroid elements, often considered peculiar to the scalp, were found in other anatomical sites, including the distal parts of the extremities.

HISTOGENESIS OF MYOSITIS OSSIFICANS. Lent C. Johnson (by invitation), Washington, D. C.

Abstract. Seventy cases of myositis ossificans were studied for correlation of duration with changes in the histologic picture. A zonal pattern was apparent in almost all lesions and was present invariably during the first 3 months. In the earliest lesions there was an outer zone of damaged muscle, a broad intermediate zone of gelatinous myxomatous tissue, and frequently a central focus of hemorrhage and débris. The intermediate zone quickly differentiated into two layers: The outer layer produced a matrix of chondroid and osteoid character and showed great cellular activity resembling the picture seen in bone sarcoma; later, cellular activity subsided, leaving an outer shell of mature cortex-like bone with a subjacent layer of cancellous bone. The inner layer of the myxomatous tissue was composed of vascularized sheets of spindle cells with the subsequent development of giant cells, so that histologically this zone simulated a malignant giant cell tumor of bone. Later the giant cells disappeared and the sarcoma-like spindle cells differentiated into an areolar connective tissue which at times suggested fibrous dysplasia. Gradually this connective tissue retracted from the center to leave a cavity which tended to enlarge. The end-stage may be a hollow sphere or tube of bone, lined by velvety, relatively acellular, loose connective tissue.

In general, the histologic appearance of all lesions during the first month strongly suggested a malignant neoplasm. During the second month, about half of the lesions continued to appear malignant, while the rest appeared benign. With very few exceptions, all lesions after the third month could clearly be identified as benign.

A similar zonal pattern with gradual transformation from a process which appeared histologically malignant to one that was clearly benign could be seen in the so-called "subperiosteal giant cell tumor" or "ossifying periosteal hematoma." In the majority of these lesions there was evidence that damaged muscle had participated in the process, thus indicating that many were a form of myositis modified by close association with the periosteum.

A study of myositis ossificans in its earliest manifestations and of the changes in skeletal muscles following crushing injury suggested that the proliferating spindle cells which produced the myxomatous pattern and matrix probably originated from the sarcolemmal sheaths of the partially damaged muscle. In 3 cases, lesions with many of the features of early myositis ossificans tended to behave as sarcoma, with recurrence or apparent invasion. In these cases the zonal pattern of growth was greatly disrupted and the malignant appearance of the cells persisted far beyond the time when the change to a benign form had occurred in the rest of the cases in this study.

SYMPOSIUM ON BONE DISEASES

SKELETAL REFLECTIONS OF DISORDERED METABOLISM.* Henry L. Jaffe, New York, N. Y.

THE SKELETAL CHANGES IN ALBRIGHT'S DISEASE. H. Edward MacMahon, Boston, Mass.

Abstract. In 1937, Albright, Butler, Hampton, and Smith described a syndrome now known as Albright's disease. This was characterized by the presence of multiple bone lesions, areas of skin pigmentation, and precocious puberty in females. They suggested the term "osteitis fibrosa disseminata" to distinguish the skeletal changes in this syndrome from those associated with hyperparathyroidism. A year later Lichtenstein, in stressing the already confused nomenclature of bone diseases, introduced the term "polyostotic fibrous dysplasia" to designate what he considered to be the same bone lesion. Lichtenstein emphasized that the specific changes in the bones can occur alone, and usually constitute the sole disorder, but he admitted that the same bone changes could be found in association with skin pigmentation, precocious puberty, and other abnormalities. Since then the term polyostotic fibrous dysplasia has become modified to "fibrous dysplasia of bone," but in this metamorphosis the original conception as a disease of bone has become greatly expanded, so that cases formerly considered as instances of Albright's syndrome have now come to be regarded as "florid" or "full blown" expressions of this elementary skeletal disorder. Naturally this has created somewhat of a dilemma both in terminology and in interpretation, for it does seem to be somewhat unreasonable to use the same term to denote on the one hand a disease that may be characterized by nothing more than a solitary bone lesion and, on the other hand, to designate a disease that can involve every system in the body—even though a specific skeletal lesion may be common to all cases.

I have had the opportunity to study the skeletal system of a girl, 9 years of age, who died with the clinical diagnosis of Albright's syndrome. Because the study of this child during life by Albright and many others from the age of 6 months to her death was in large measure responsible for the conception of Albright's syndrome, it seems justifiable in a Symposium on Bone Diseases such as this, to report the skeletal findings.

Instead of a single type of skeletal lesion, there were three separate and distinct bone changes. First, and most striking, was the osteitis fibrosa disseminata or fibrous dysplasia that by x-ray and also by autopsy, as far as could be determined, involved every bone in the body. Secondly, there was a disturbance in epiphyseal growth involving both bone and cartilage; and lastly, there was widespread bone atrophy.

* By invitation of the Council.

The first of these, which in some respects was the most striking feature of the entire syndrome, showed many of the gross and histologic features of both fibrous dysplasia and osteitis fibrosa cystica associated with hyperparathyroidism. The bones were deformed, lighter in weight, softer in consistency than normal, and could be easily broken. There were signs of old and more recent fractures. In many bones the normal pattern of cortex and medulla was partially or completely replaced by areas of firm, rubbery, gritty, gray fibrous tissue. The histologic changes in such areas were characterized by a progressive replacement of normal bone and bone marrow by fibrous tissue, cartilage, osteoid tissue, myxomatous tissue, and new bone.

The second type of bone lesion was centered in and about the epiphyseal cartilage. In some areas the epiphyseal lines were nearly normal, but in many places they were ragged and uneven and blunt tongues and long finger-like projections of cartilage extended deeply into the adjacent diaphysis. In other areas segments of epiphysis had completely disappeared, allowing the epiphyseal and diaphyseal bone to fuse prematurely. Still another change involving cartilage was a cessation of skeletal growth with the apposition of well formed bone plates in areas where active endosteal cartilaginous osteogenesis should at her age be taking place.

The third change affecting the skeletal system was characterized by atrophy and osteoporosis. This was most clearly seen in the cortex in areas entirely free of fibrosis. In such fields dilated haversian canals were filled with actively hematopoietic marrow.

Of these three changes, the fibrous dysplasia was by far the most striking. It is of particular interest that there were many histologic features in common with both fibrous dysplasia and hyperparathyroidism. At first, this might suggest a unique condition, but the finding of four enlarged parathyroid glands composed of an almost solid sea of cells, of which many were of the water-clear type, could possibly explain this unusually complex and apparently "combined form" of bone lesion.

Discussion

(Dr. Louis Lichtenstein, New York, N. Y.) There are so few autopsied cases of fibrous dysplasia of bone on record, or of Albright's syndrome, if you will, that this contribution of Dr. MacMahon's is a very important one. It was extremely interesting, therefore, to follow the whole gamut of skeletal changes which he described, though it is a pity that the limitation of time caused him to rush through them so quickly. In the main, the skeletal findings which Dr. MacMahon described are in accord with the changes Dr. Jaffe and I have observed in less severe cases of fibrous dysplasia affecting one, several, or many bones, with or without associated pigmentation and various endocrine disorders. The features suggesting hyperparathyroidism, on the other hand, we have not encountered, but then we have not had the fortunate opportunity of examining any of these extremely severe cases at autopsy. I should like to ask one specific question in regard to the distribution of cartilage foci within the interior of the affected bones: Were they all found in the neighborhood of the plate regions, so one might assume they represent fragments of pinched-off cartilage, or were some of them found well down the marrow cavity, so that one would have to explain them on some other basis, such as an integral feature of the skeletal dysplastic condition? We were already aware of the presence of such cartilage foci when we published our first paper on fibrous dysplasia of bone and, indeed, suggested at that time that this disorder represented a developmental anomaly in a sense related

to skeletal enchondromatosis, and that the two conditions might be regarded as first cousins.

(Dr. Joseph E. Pritchard, Montreal, Canada) Were there any kidney changes or any renal insufficiency in this case?

(Dr. MacMahon) In answer to Dr. Lichtenstein's question about the distribution of cartilage, there were large islands of hyaline cartilage in the immediate vicinity of epiphyseal lines, but there were larger islands far removed from these areas, particularly at sites of earlier fractures. Perhaps most interesting were the islands of cartilage within the substance of the calvarium which, being a membrane bone, is ordinarily free of cartilage. Cartilage adjacent to the epiphysis appeared to be of epiphyseal origin, whereas cartilage forming in the shaft seemed to arise locally, being formed directly by endosteal fibrous tissue or indirectly through the formation of a chondroid mucinous ground substance. The fate of the cartilage was as interesting as its origin. Some islands composed of hyaline cartilage showed no sign of change; some showed maturation, marginal calcification, and enchondral ossification; others showed direct metaplasia to bone and still others showed marginal resorption by growing fibrous tissue and multinucleated chondroclasts.

In answer to Dr. Pritchard's question, the kidneys were well preserved and showed no sign of renal decompensation. Like so many of the organs of this child, the kidneys did show developmental anomalies, but they were relatively insignificant and consisted of a few minute pyramidal cysts.

NEUROFIBROMATOSIS AND ITS RELATIONSHIP TO CERTAIN DISEASES OF BONE.

Ernest Aegerter, Philadelphia. Pa.

Abstract. Neurofibromatosis is a condition characterized by multiple nodules of fibroblastic hyperplasia of nerve supportive tissue. It is variably associated with a number of lesions in bone: scolioses, asymmetric hypertrophy, and focal neurofibromas usually misdiagnosed as bone cysts. It or its associated conditions accompany congenital pseudo-arthritis and fibrous dysplasia in a relatively high incidence.

The contention that fibrous dysplasia is a manifestation of neurofibromatosis has been ably refuted by Jaffe. In this paper the author challenges the widespread belief that congenital pseudo-arthritis is caused by a neurofibroma growing in and destroying bone substance. The clinical behavior of pseudo-arthritis and its gross and microscopic characteristics are cited to show that it is a dysplasia of cortical bone and callus formation.

It is suggested that all the above conditions, including neurofibromatosis, are dysplasias rooted in defective mesenchymal germ plasm. The character of each lesion is apparently conditioned by the modifying influence of its tissue environment. Treatment, especially of pseudo-arthritis, should be designed on this basis. A described successful method appears to substantiate this impression.

PATHOLOGIC CHANGES FOUND IN CURETTINGS FROM THE HEAD OF THE FEMUR IN 31 CASES OF OSTEOCHONDROITIS DEFORMANS JUVENILIS (LEGG-CALVÉ-PERTHES' DISEASE). Samuel R. Haythorn, Pittsburgh, Pa.

Abstract. The materials examined were curettings from the head and neck of the femur in 31 cases of Legg-Calvé-Perthes' disease. In 1939 P. B. Steele reported a series of cases on which he had operated to hasten healing in Perthes' disease by opening the hip joint, drilling into the head of the femur, removing the diseased contents, and filling the cavity with curls of bone from the femoral neck. The purpose was to remove débris, support the head with bone grafts, and stimu-

late healing. Thirty-one of 32 clinical cases showed practically the same pathologic changes and varied only in the degree and amount of change. Constant findings were aseptic necrosis or necrobiosis, compression of the various bony elements, and incomplete healing processes which were apparently going on concurrently and to little purpose. The necrosis involved all of the tissues present to varying extents and included destruction of marrow with bony trabeculae recognizable, but without nucleated osteocytes or osteoblasts. Cartilage showed fragmentation, degeneration, and imperfect ossification. The larger bits of cartilage had lost nuclear polarity and the chondrocytes were arranged as constellations of many patterns. Side by side with the degenerated areas were bizarre islets of healing including simple fibrous replacements of marrow stroma which was infiltrated sparsely with lymphocytes and occasional leukocytes. The connective tissue tended to form miniature cysts with foreign body giant cells in their walls. Areas of osteogenesis and osteoclysis were jumbled together and the normal line of ossification of the epiphyseal cartilage was lost. Vascular changes were on the progressive side. Disproportionately larger arterioles with proliferative endarteritis were sometimes seen. The lesions are interpreted by the author as being due primarily to nutritional disturbances associated with vitamin and growth hormone deficiencies. The changes weaken the resistance of the femoral head, and the superimposed minor injuries of weight bearing and frustrated attempts at repair complete the picture.

HISTOCHEMICAL STUDIES ON CARTILAGE AND BONE. Richard H. Follis, Jr., and (by invitation) Morgan Berthrong, Baltimore, Md.

Abstract. Although numerous isolated observations on certain histochemical reactions in cartilage and bone have been recorded, no integrated studies have been carried out. We have felt it desirable to determine the normal histochemical pattern in cartilage and bone in order to prepare for a study of changes in the skeleton produced experimentally by means of vitamins and hormones and bone disease in man.

Observations have been carried out on fresh, free-hand tissue slices and undecalcified sections fixed in various ways. The distribution of the following substances has been studied: cytochrome oxidase, succinic dehydrogenase, citric acid dehydrogenase, alkaline phosphatase, iron, glycogen, mucopolysaccharides, desoxyribose nucleic acid, ribose nucleic acid, neutral fat and ascorbic as well as oxidation-reduction potentials. Certain of the results are as follows. Succinic dehydrogenase is present in cartilage, being particularly prominent in cell nuclei. We have not been able to estimate quantitative differences in relation to maturation of cartilage cells. The osteoblast is rich in cytochrome oxidase as indicated by the nadi reagent. Cartilage does not appear to contain this enzyme. Sections stained by toluidine blue show intense metachromasia of the cartilage matrix material as well as the organic portion of bone, osteoid. Glycogen can be demonstrated in osteoblasts and osteocytes as well as in cartilage cells. The only structures giving a positive Feulgen reaction are the nuclei of cartilage and bone cells. This may be destroyed by incubation with desoxyribonuclease. Colchicine brings out the striking proliferative activity of growing cartilage.

FIBROUS DYSPLASIA OF THE MANDIBLE AND MAXILLA. A. R. Crane and (by invitation) J. R. Wolgamot, Philadelphia, Pa.

Abstract. Eleven cases of monostotic fibrous dysplasia, 6 of the mandible and 5 of the maxilla, have been studied. These show the same feminine predominance as the polyostotic form, 8 occurring in females. Four were in Negroes. Patients

varied from 9 to 56 years of age at the onset. Swelling was the presenting symptom in each case. A history of trauma was given by 4 patients, but this was insignificant in degree. Two cases showed abnormal but slight skin pigmentation. Sexual precocity, genital abnormalities, or precocious skeletal growth were not present. Radiologically, there was focal rarefaction of the mandible or maxilla or increased density in the maxillary sinus. Histologically, all showed fibrous replacement of old bone with membranous new bone formation. Osteoclastic giant cells were present in relation to bone destruction or production, and were a minor part of the picture. Rare, small cystic zones were present in some cases; cartilage was absent. Similar histologic changes were seen in cases of lues, radiculodental cysts, benign giant cell tumors, myositis ossificans of the masseter muscle, and epulides, but these had other identifying features. Ossifying fibroma was not distinguishable from the cases of fibrous dysplasia. Chemical studies (calcium, phosphorus, and alkaline phosphatase) were normal in every case. Patients were treated by curettage or simple re-establishment of normal contour, and are living from a few weeks to 10 years without progression of the abnormality. Lesions of other bones or fractures have not occurred. These findings suggest that there is no line of distinction between monostotic fibrous dysplasia, polyostotic fibrous dysplasia, and ossifying fibroma.

CHONDROMYXOID FIBROMA OF BONE. Louis Lichtenstein, New York, N.Y.

Abstract. This paper deals with a peculiar benign tumor of bone, which seems not to have been generally recognized in the past as a distinctive neoplasm, although it appears likely that single instances of it have been reported as enchondroma or myxoma and their malignant counterparts. Our interpretation of the lesion is that of a peculiarly differentiated, connective tissue tumor exhibiting, in the course of its evolution, certain chondroid and also myxoid traits which hallmark the lesion cytologically. It is composed basically of cells lying loosely in a myxoid intercellular matrix which, as the tumor matures, may undergo substantial collagenization. The tissue of any particular tumor may also come to simulate cartilage tumor tissue in some or many fields; and, in its gross appearance, it likewise bears a certain resemblance to cartilage. The presence of smaller or larger numbers of tumor cells exhibiting nuclear atypism may cause the lesion to appear more ominous than we know it to be, explaining why it may come to be overdiagnosed as a malignant tumor, and particularly, as chondrosarcoma.

Our experience with this tumor to date comprises 8 cases, and we have encountered it thus far only in one or another bone of a lower limb, and specifically, in the femur, the tibia, and some of the foot bones. Within the femur or tibia, the lesion was found consistently in the metaphyseal area adjacent to the knee joint. Most of the patients were adolescents or young adults, though some were older. The lesion, as a rule, evolves slowly and is often of some months' or even a few years' standing before surgical intervention is sought. The roentgenographic picture has a certain distinctiveness, at least when the lesion is in a long bone and has attained appreciable size, although its differentiation at times from bone cyst, enchondroma, or a focus of fibrous dysplasia may be difficult without tissue examination. The tumor is apparently entirely benign and does not tend to recur after mere curettage, even without supplementary radiation. While the tumor is not a particularly common one, its recognition is of some importance in that, pathologically, it may readily be mistaken for a sarcoma, and, as such, treated more radically than is necessary.

Discussion

(Dr. V. H. Cornell, Washington, D.C.) I would like to ask how Dr. Lichtenstein tells them apart.

(Dr. Lichtenstein) Tells what apart?

(Dr. Cornell) The ones that are going to be benign, and those which are going to be malignant. What I mean is this. You have said many of these would be mistakenly diagnosed, and I agree with you. We usually consider myxomatous tissue rather a dangerous thing to play around with. Naturally your history of the cases indicates they are benign. Were there mitoses? Is all the tumor such as we saw on the screen, or is some more abnormal and the cells more irregular? Is there anything to make the differentiation between the benign and the malignant?

(Dr. Joseph E. Pritchard, Montreal, Canada) Is this tumor any different than the common chondromyxoma? Certainly some of them are excentric, but it seems to me that the appearance is very much the same, only some of them are endosteal and some periosteal.

(Dr. Lichtenstein) In reply to Dr. Cornell's question, I venture to state that if you were to see an instance of this particular tumor for the first time or perhaps even for the second time, you might very well fall into the error we ourselves did when we first encountered this lesion. As I remarked, the first two cases which Dr. Jaffe and I encountered were called chondrosarcoma initially. Indeed, it was only on reviewing all the relevant lesions against a background of genuine chondrosarcoma material that we appreciated distinct cytologic differences. Furthermore, we discovered in our follow-ups that the patients in question showed no tendency to local recurrence even after simple curettement. In selecting the photomicrographs I attempted to illustrate various cytologic features of the lesion, and the over-all impression thus created may have been that of a lesion of rather variegated appearance. Actually, the tumor under discussion has a distinctive cytologic picture which is rather easy to recognize if one is familiar with it. To one who is not familiar with it, the cytologic picture may be misleading and create a false impression of malignancy. If this presentation is instrumental in preventing a half dozen or more needless amputations, I think it will have served a useful purpose. We know that the tumor is benign on empirical grounds, for it seems reasonable to assume that when a lesion is treated by simple curettement and does not recur during the ensuing 6 or 7 years, that it is clinically benign, no matter what its cytologic character may be. In other words, we must judge the tumor by what it does, rather than by what it looks like.

As to the question raised by Dr. Pritchard, there are distinct gross and microscopic pathologic differences between the appearance of this tumor and the common garden variety of enchondroma, although it is true that roentgenographically one cannot always distinguish them with assurance.

EWING'S TUMOR OF BONE. Horace K. Giffen, Youngstown, Ohio.

Abstract. In 1922 Ewing described a primary malignant bone tumor which is found in children mainly. He called it "diffuse endothelioma of bone." The first evidence of the disease is usually associated with fever, pain, tenderness, and occasionally leukocytosis. The primary site is often in the diaphysis of a long bone. There is marked osteolysis with spread along the shaft, cortical destruction, expansion, and perforation. Through the blood stream other bones, oc-

casionally regional lymph nodes, and eventually the lungs may be involved. There is marked temporary response to radiation. Current opinion favors considering these tumors as malignant reticulomas or reticulosarcomas.

Two examples of this clinical entity follow. The first was a 13-year-old boy, who began to complain of pain in the upper right thigh in mid-July of 1945. While playing baseball on August 9th he fractured his femur without direct trauma. Roentgenography and biopsy showed endothelial myeloma. Radiation gave temporary relief, but by January of 1946 headaches had begun. During the following months spread of tumors progressed until his death on September 17, 1946.

The second case was a 16-year-old girl who complained of her left shoulder in May, 1946. For about 2 months a chiropractor treated the shoulder for "dislocations." Admitted to our hospital in mid-July, biopsy and roentgenologic examination revealed endothelial myelomatous involvement of the left scapula. No other bony lesions could be found at that time. But by September 13th there was evidence of metastasis in the 3rd lumbar vertebra. During the following weeks she became emaciated, anemic, febrile, and blind in the left eye. She died on November 11, 1946, or about 6 months after the first symptoms.

Discussion

(Dr. Osborne A. Brines, Detroit, Mich.) Was there any adrenal involvement in either one of these cases?

(Dr. Alfred Angrist, Jamaica, N.Y.) The question of neuroblastoma I think deserves some stressing here, because sometimes the primary can be rather small and still originate from the usual or an unusual site for the neuroblastic tumors or the less differentiated forms of ganglion cell tumors. It has been my experience that though Ewing's tumor is a common surgical diagnosis, this diagnosis is uncommon at autopsy, and this has been true even when the diagnosis has been established by eminent authorities. We have established a rule that the surgical diagnosis of Ewing's tumor in a youngster requires ruling out a neuroblastoma, and in an adult requires ruling out a bronchogenic carcinoma. In every case, and we have had about a dozen in which the diagnosis of Ewing's tumor was made clinically, autopsy showed one or the other of these tumors.

(Dr. Giffen) The first case did show a metastatic lesion in one of the adrenals. It was in the adrenal cortex with no involvement of the medulla. We looked in vain for other lesions of the sympathetic nervous system or any other lesion which might have been the primary tumor. The first case had multiple small lesions in both lungs, small intestine, pancreas, mesenteric lymph nodes, both kidneys, one adrenal cortex, dura, and outer cerebral cortex. The other case showed lesions in the soft tissues of both lungs, pancreas, dura, leptomeninges, and outer cerebrum. I do not feel that in either of these cases there was any evidence of primary tumor elsewhere; we certainly thought of possible primary tumor elsewhere, but we could not find it.

PLASMA CELL MYELOMA ASSOCIATED WITH HIGH CONCENTRATION OF PLASMA LIPOPROTEIN. R. M. Hill (by invitation), R. M. Mulligan and (by invitation) S. G. Dunlop, Denver, Colo.

Abstract. The patient was a white female houseworker, 55 years old, with severe sensitivity to cold for 7 years previous to admission on August 7, 1945. Exposure to cold resulted in severe erythema and urticaria of the exposed parts followed by the development of shallow ulcers.

She sustained a fracture of the left humerus 1 year before admission. Examination disclosed a temperature of 99.6°C ., blue mottling of the fingers and toes, and pain and tenderness on motion of the left shoulder. The urine was negative. Her blood coagulated when withdrawn from a vein into a test tube and reliquefied on warming. Blood hemoglobin was 4 to 5 gm. per cent; erythrocytes, 1,950,000 to 3,250,000 per cmm.; and leukocytes, 6,700 to 7,200 per cmm., with a differential of 30 to 48 per cent segmented neutrophils and 50 to 62 per cent lymphocytes. Serum calcium was 10.4 mg. per 100 cc.; phosphorus, 4.2 to 7.1 mg. per 100 cc.; and alkaline phosphatase, 2.4 Bodansky units. Roentgenograms revealed expansion and destruction of the head of the left humerus. On August 31st incision for biopsy of the lesion in the left humerus was followed by hemorrhage. On September 9th the entire floor of the mouth became so swollen that dyspnea supervened and the patient died with a fever of 107.8°C . at 12:30 p.m.

A sample of freshly drawn heparinized blood, centrifuged at room temperature (25°C .) at 2,000 r.p.m. for 20 minutes, separated into three layers; an upper layer of apparently normal plasma, a middle pearly white layer solidifying on cooling, and a lower layer of cells. In a sample of 7.5 cc., the volumes of these layers were 3 cc., 2.8 cc., and 1.7 cc. The pearly white middle layer (hereinafter referred to as a cryoprotein) was 37 per cent of the volume of the blood and gave the characteristic reactions of a protein. Four subsequent blood samples showed the same phenomenon. After blood transfusion, blood samples failed to show the same change, since the entire plasma solidified on cooling and, by diluting and cooling the plasma, the cryoprotein separated as a flocculent precipitate. On August 11th, total plasma proteins were 7.2 gm. per 100 cc. with albumin 2.2 gm. per 100 cc., and globulin plus fibrinogen 5.0 gm. per 100 cc. Blood nonprotein nitrogen was 29 mg. per 100 cc. and cholesterol 146 mg. per 100 cc. On August 24th, the total plasma proteins were 10 gm. per 100 cc., with albumin 1.8 gm. per 100 cc., globulin 6.5 gm. per 100 cc., and fibrinogen 1.7 gm. per 100 cc. The protein content of the cryoprotein layer was 13.1 gm. per 100 cc. The top layer revealed a total protein of 7.1 gm. per 100 cc., with albumin 2.1 gm. per 100 cc., globulin 4.5 gm. per 100 cc., and fibrinogen 0.5 gm. per 100 cc. On August 28th, following blood transfusion, the total plasma protein was 18.1 gm. per 100 cc., with albumin 2.1 gm. per 100 cc., globulin 9.8 gm. per 100 cc., and fibrinogen 6.2 gm. per 100 cc. At 38°C . the viscosity of the plasma of the patient was 5.3 times that of the average normal, rose rapidly with falling temperature, and reached infinity at 32°C . The cryoprotein was purified by 10 reprecipitations from distilled water, finally brought into aqueous solution, and precipitated by the addition of an equal volume of 95 per cent ethyl alcohol. After standing for 3 days at 4°C . in the icebox, needle-like crystals proved to be cholesterol ester. Microbiologic assay of the amino acids of the purified protein was compared with the analyses of the amino acids in normal plasma globulin and albumin.

Autopsy revealed plasma cell myeloma involving the bone marrow, chiefly of the left humerus, scapula, and clavicle, but also of the ribs, vertebrae, sternum, and skull, the spleen, and a mesenteric lymph node; edema, hemorrhage, and focal atelectasis of the lungs; a gelatinous cast in the bronchus to the lower left lobe; focal acute pharyngitis; clinical Ludwig's angina; and a recent unhealed incision of the left arm. The parathyroid glands, heart, liver, kidneys, brain, and spinal cord were normal. The bone of the head and neck of the humerus was extensively destroyed by massive proliferation of cells having the structure of plasma cells, chiefly in the plasmablast stage. The cells exhibited abundant basophilic cytoplasm and frequently an eccentric position of

the nuclei, which were large and rounded, contained much fine heavily stained chromatin, one or more prominent nucleoli, and numbered one to four to a cell.

BONE TUMORS COMPOSED OF ATYPICAL AMYLOID. William H. Bauer (by invitation) and J. F. Kuzma, St. Louis, Mo., and Milwaukee, Wis.

Abstract. The necropsy of a 59-year-old male who died of purulent meningitis revealed destructive tumors of the sphenoid, the left 9th rib, and the 9th dorsal vertebra. Roentgenographically the other bones failed to show any changes. The clinical record revealed the absence of Bence-Jones proteinuria, hyperproteinuria and albuminuria. The sedimentation rate was normal, peripheral blood appeared unchanged, there was no excessive formation of rouleaux, no "greasiness" of the blood smears, and the calcium-phosphorus metabolism was unaltered. The liver, spleen, kidneys, and intestine microscopically and macroscopically were unchanged.

The lesions of the aforementioned bones consisted of numerous bodies of an atypical amyloid with an abundance of giant cells. Only the sections through the sphenoid contained a scattering of atypical plasma cells and a few lymphocytes. The microscopic findings did not conclusively substantiate the diagnosis of multiple myeloma. The atypical amyloid differed from typical amyloid not only in its distribution, but also by its indistinct reaction to methyl violet and Congo red. Furthermore, it not infrequently displayed a smooth transition into osseous tissue and even appeared more densely calcified than the bone trabeculae. These findings suggest that its chemical composition differed from that of typical amyloid. Microscopically, the predominant formation of the atypical amyloid was by fusion of the protoplasm of degenerated bone marrow cells and giant cells. Secondly, intercellular protoplasm-like globules of unknown origin participated in its deposition. Whether we were dealing with atypical multiple myeloma or the result of a chronic infectious process cannot yet be answered.

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BERYLLIUM PNEUMOCONIOSIS. Walter W. Jetter, Boston, Mass.

Abstract. Beryllium has come into recent importance because of its use in preparing fatigue-resistant copper alloy and fluorescent lamp powder. Aside from an acute and apparently spontaneously subsiding episode of chills and fever similar to "metal fume fever," pulmonary reactions to beryllium may fall into either of the following two types.

The first type of reaction may occur after several weeks or months of exposure to dusts or fumes containing beryllium fluoride or oxyfluoride arising during extraction or processing of the ore. The pathologic lesion in the lungs consists in the activation of alveolar macrophages which then may accumulate in the intrapulmonary air spaces. The usual course is spontaneous remission but occasionally distortion and obliterating fibrosis of lung parenchyma may lead to severe pulmonary insufficiency. The deleterious process has been attributed to the acid fluoride and oxyfluoride radicals rather than to beryllium *per se*.

The second type of pulmonary reaction is a peculiar form of pneumoconiosis, "beryllosis" or "beryllium granulomatosis," which develops insidiously and is chronic in its course. Beryllium has been incriminated in most or nearly all of the cases. In our experience, the disease has occurred among workers exposed to fluorescent lamp powder (zinc manganese beryllium silicate) although similar cases have been reported in the manufacture of beryllium alloys. The lungs in fatal cases have shown extensive nodular and irregular fibrosis. Cor pul-

monale is characteristically present and it is apparent that right heart failure has contributed significantly to death. Microscopic examination indicates that the initial lesion is an intra-alveolar collection of phagocytes. Foreign body giant cells are prominent in this inflammatory process and occasionally they may be seen to contain, or to be in relation to, peculiar basophilic bodies, sometimes referred to as "conch shells." As the granulomatous lesions expand peripherally they tend to merge with one another so that widespread areas may become involved. Concurrent fibroblastic infiltration and resultant scar formation leads to extreme distortion and contraction of contiguous lung tissue. Scarring and hyaline change in a discrete nodule may result in a structure resembling the silicotic nodule. In contrast to silicosis, complicating tuberculous infection has not been observed. The final picture is an extraordinary mixture of active focal and diffuse granulomatous inflammation in combination with dense nodular and irregular fibrosis and hyalinization. The tracheobronchial lymph nodes are enlarged and show varying degrees of active inflammation and connective tissue scarring. Nodes completely replaced by hyalinized fibrous tissue may be encountered. The occurrence of granulomatous lesions in liver and spleen may indicate that the toxic agent or agents are disseminated by the general circulation.

Another prominent feature in beryllium pneumoconiosis is severe, diffuse, obliterating pulmonary endarteritis. The resultant decrease in the capacity of the pulmonary vascular bed may be largely responsible for the occurrence of cor pulmonale and ultimate right heart failure.

Discussion

(Dr. Donald A. Nickerson, Salem, Mass.) These cases are interesting to us, because it was in 1942 that Connolly recognized this condition, and there are now about 60 cases which have roentgenologic and clinical evidence of the disease. Perhaps the most interesting thing about this is that after a new plant was opened the content of beryllium in the phosphor was changed. Up to that time this particular company had used 12.9 per cent beryllium, whereas the new manufacturer used 2.5 per cent. After this change, as far as we know, only 1 or 2 cases have developed.

Another interesting thing is that I have seen 3 cases of injury to the skin. In those sites we have found changes exactly identical with those in the lung, and in those lesions beryllium has been found. Perhaps we will see more of it, because one of these cases was in a young boy who was using a burned-out tube as a baseball bat.

In the cases I have seen the ash content of the lung has shown in all cases levels of 5 μ g. of beryllium per 100 gm. of lung tissue.

(Dr. Jetter) I wish to thank Dr. Nickerson for his interesting comments. The evidence indicates the likelihood of a causal relation in the production of this chronic pulmonary disease by inhalation of beryllium-containing fluorescent powders. Positive proof awaits the production of the disease following experimental exposure in laboratory animals.

ENLARGEMENT OF THE BRONCHIAL ARTERIES AND THEIR ANASTOMOSES WITH THE PULMONARY ARTERIES IN CHRONIC PULMONARY DISEASE. Averill A. Liebow, and (by invitation) Milton R. Hales and Gustaf E. Lindskog, New Haven, Conn.

Abstract. In 14 of 17 lung specimens from patients prepared as casts by the vinylite corrosion technic, great enlargement of the bronchial arteries and numerous anastomoses of these vessels with the pulmonary arteries were observed. The

communications were multiple and usually occurred about the bronchiectatic sacs which involved the fourth order branches of the segmental bronchi, and beyond. In half of the specimens the anastomoses equalled or exceeded a diameter of 1 mm. The enlargement of the bronchial vessels probably occurs in response to the increased demand for oxygenated blood in the granulation tissue during the course of the organizing pneumonia that usually precedes bronchiectasis, and in the hypertrophied muscle and hyperplastic lymphoid tissue that are often observed. The anastomoses may represent persistent communicating channels originating in granulation tissue that receives vessels from both the bronchial and pulmonary arterial systems.

So large and numerous are the anastomoses as to suggest that they have physiologic importance: (a) In shunting blood away from the diseased tissue into relatively intact parenchyma, where the blood pressure is presumably lower; (b) in producing general hypertension in the pulmonary circulation.

Discussion

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder if the authors have had the opportunity to correlate such anastomoses with the state of hypertrophy of the right heart. My second question is fortuitous if the technic of these investigations has been published, but if not, will Dr. Liebow describe it? The preparations are the most exquisite I have seen in a long time.

(Dr. Liebow) We have had only one autopsy specimen from a patient with severe bronchiectasis. This was demonstrated on the screen. In that case there was great enlargement of the right side of the heart, but this was associated not only with bronchiectasis but with a very striking bilateral pulmonary emphysema. One might expect that the burden of the right heart would be increased if there were a diffuse bilateral bronchiectasis. In unilateral bronchiectasis the pressure in the pulmonary artery would be increased only if there were a back flow within the pulmonary artery of the involved side from the region of the anastomoses with the systemic bronchial circulation into the proximal end. Indeed it is possible that this may occur since the anastomoses are so large and so numerous. Catheter studies will have to be done to determine whether or not "back flow" occurs. In the absence of back flow, unilateral bronchiectasis should not produce pulmonary arterial hypertension since the pulmonary capillary bed of one lung is sufficient to accommodate the entire cardiac output without rise in pulmonary arterial pressure, as Cournand has demonstrated.

In answer to Dr. Angrist's second question, the technic has been published in the Bulletin of the International Association of Medical Museums, and there is a demonstration of the method among the exhibits of that Association.

THE RELATIONSHIP OF FIBROCYSTIC DISEASE OF THE PANCREAS TO A DEFICIENCY OF SECRETIN. Archie H. Baggenstoss, and (by invitation) Marschelle H. Power and John H. Grindlay, Rochester, Minn.

Abstract. In a recent study of the pancreas in uremia a remarkable degree of dilatation of the acini, flattening of the lining epithelial cells, and inspissation of secretion were observed in approximately 45 per cent of the cases. A high incidence of the same lesion was observed also at necropsy in cases of carcinoma of the stomach, obstruction of the small intestine, chronic ulcerative colitis, and sepsis. It has been suggested by one of us that the following factors are important in the pathogenesis of this lesion: (1) inhibition of the type of pancreatic secretion normally stimulated by secretin, (2) nervous stimulation of the pancreas, leading

to depletion of zymogen granules and formation of a thick, viscid pancreatic juice, (3) dehydration, resulting in inspissation of the juice and the development of intrinsic obstruction, and (4) malnutrition (protein deficiency), resulting in a failure of reparative protein synthesis in the cells of the pancreatic acini.

Because of the similarity of the histologic picture to that observed in the early stages of fibrocystic disease of the pancreas, it was suggested also that a congenital deficiency of secretin was responsible for the latter disease. This hypothesis is based upon the concept first suggested by Blackfan and Wolbach and supported by Farber and Andersen that fibrocystic disease of the pancreas is the result of an abnormally thick and inspissated acinar secretion. If one postulates an inhibition or an absence of stimulation of the pancreas by secretin, the only stimuli to secretion would be nervous and that recently ascribed to pancreozymin. Such an imbalanced stimulation of the pancreas would result in a thick viscid juice which might become inspissated, obstruct the ductules and acini, and lead to atrophy and fibrosis. Stimulation of the pancreas by secretin has been described as causing a flow of alkaline fluid which serves to flush the alveoli, to thin the juice rich in organic material and to sweep it along the ducts. Any satisfactory explanation of the pathogenesis of fibrocystic disease of the pancreas must also explain the disturbances which occasionally occur in the secretions of the liver and intestines. There is good evidence that, normally, secretin stimulates the secretion of bile and the succus entericus. If stimulation by secretin did not occur, the obstruction of the small bile ducts and intestinal glands in these cases might also be explained as the result of the production of an abnormally thick inspissated secretion.

In order to test this hypothesis it was necessary to determine (1) if secretin could be obtained from specimens of the duodenum and small intestines at autopsy, and (2) if it were absent in patients dying of fibrocystic disease of the pancreas. Up to the present we have been able to extract secretin (S) according to the methods outlined by Ivy and his co-workers from specimens of the upper part of the intestinal tract of 17 of 18 adults. The single failure occurred in a case of chronic intestinal obstruction of 4 weeks' duration. The extraction of secretin in the other cases was successful whether it was carried out immediately or after the specimen had been frozen for varying intervals of time up to 39 days. Secretin could be extracted from specimens kept unfrozen as long as 14 hours post-mortem. We have been able to extract secretin from the upper part of the intestinal tract of all children studied except premature and newborn infants and one child with fibrocystic disease of the pancreas. The specimen from the child with fibrocystic disease was sent to us through the courtesy of Dr. J. P. Simonds and Dr. Philipsborn of Chicago. We are hoping to obtain a sufficient number of specimens to test our hypothesis thoroughly before arriving at any conclusions. For the present we should like to suggest that fibrocystic disease of the pancreas is the result of a congenital deficiency of secretin. This deficiency may be the result of a congenital absence (relative or complete), a defect in the mechanism of its release, or its destruction by abnormal amounts of secretinase. This preliminary report of our work is concerned only with the first possibility.

Discussion

(Dr. William R. Platt, Louisville, Ky.) Does this deficiency of secretin have anything to do with the production of meconium ileus in the newborn?

(Dr. Betty B. Geren, St Louis, Mo.) How does Dr. Baggenstoss postulate that the lack of secretin would produce changes in the salivary glands or bronchial mucous glands as seen in cases of fibrocystic disease?

(Dr. Baggenstoss) I think the fact that meconium ileus occurs also indicates

a deficiency in secretin. Normally, secretin stimulates the intestinal glands and increases the flow of succus entericus. If secretin were absent, it might explain the inspissated meconium that occurs in those cases. It does not explain the changes that have been described in the salivary glands. We did not observe these changes, and a number of other investigators have not been able to find them. If dilatation of salivary gland acini is an essential part of the disease, deficiency of secretin does not explain it. The changes in the bronchial glands we have ascribed to pulmonary infection.

PRODUCTION OF UNILATERAL ULCERATIVE PULMONARY PHTHISIS BY QUANTITATIVE NATURAL AIRBORNE CONTAGION.* Max B. Lurie and (by invitation) Samuel Abramson, Philadelphia, Pa.

Abstract. A fine suspension of tubercle bacilli, free from clumps, is sprayed with compressed air through a specially designed nozzle. The large particles settle out quickly. The invisible droplet nuclei are sucked into a pipe 16 feet long through a chamber in which rabbits are exposed, by the draft action of a flame at the bottom of a chimney devised by Wells. The infected air, after its incineration in the hot flame, is drawn to the outside by a fan. The concentration of the tubercle bacilli in the air respired by the rabbits is determined culturally by a modified Wells air centrifuge. Since the volume of air breathed by the rabbits in a given time can be determined, and since the number of bacillary units in a given volume of air is known, the number of bacilli to which the rabbits were exposed can also be estimated. When rabbits were killed immediately after exposure it was found that the number of bacilli cultured from the lungs corresponds closely to the number of bacilli estimated to have been inhaled.

The number of primary tubercles developed in the lungs is to some degree proportional to the number of bacilli in the air respired by the rabbits. However, there is great variation between individual rabbits inhaling the same infected air both as to the number of tubercles developed and their progression.

Inbred rabbits of high genetic resistance to tuberculosis were immunized with heat-killed tubercle bacilli and exposed to the inhalation of about 50 droplet nuclei of the virulent bovine bacilli. In some rabbits there was no tuberculosis at autopsy. Others acquired nonprogressive encapsulated cavities in one or both lungs, while still others developed a unilateral ulcerative phthisis. There was no dissemination of the disease by lymphogenous or hematogenous routes. The disease thus acquired strikingly resembled human ulcerative phthisis of the reinfection type.

Discussion

(Dr. Murray D. Angevine, Madison, Wis.) I should like to ask Dr. Lurie about the incidence of involvement of the liver and spleen in this group, or was the kidney representative of the dissemination?

(Dr. Kornel L. Terplan, Buffalo, N.Y.) I have a brief comment on the interesting experiments of Dr. Lurie. From his findings no conclusions should be drawn as to the presence or absence of tuberculous lesions in lymph nodes draining the site of reinfection tuberculosis in man. Even if these lymph nodes are grossly not conspicuously enlarged, as a rule, in microscopic examination, recent tubercles are found in the bronchomediastinal lymph nodes. Such findings sometimes are very marked, especially in older individuals, obviously in connection with considerable loss of their resistance against tuberculosis. Occasionally in such cases, even gross enlargement is seen, with more or less marked caseation of the lymph nodes

* Aided by a grant from the Commonwealth Fund.

draining pulmonary areas, the site of a progressive reinfection tuberculosis.

(Dr. Alfred Angrist, Jamaica, N.Y.) Will Dr. Lurie comment on what the photographs seem to show, as to apical localization in these rabbits, contrary to the usual site of localization in rabbits?

(Dr. Stanley H. Durlacher, Edgewood, Md.) How far down the respiratory tract do these aerosol particles extend?

(Dr. Lurie) In answer to Dr. Angevine's question, I can say that tuberculosis in the liver or spleen of rabbits under these conditions rarely occurs. Occasionally, microscopic tubercles may be seen in these organs in rabbits with a long-standing disease, but visible, macroscopic tubercles are extremely rare, whereas tuberculosis of the kidney nearly always occurs.

As to the question of Dr. Terplan, I must say that these lymph nodes have not been investigated completely. In a number of animals in which the lymph nodes were studied microscopically no tubercles were seen, but in other cases they were found.

As to the question of apical tuberculosis, the primary tubercle in the lung of rabbits may occur anywhere in the lung; however, rarely, if ever, are apical localizations seen. The lower lobe sometimes is affected, but in my experience the upper lobes of both lungs are frequently the site of the primary lesion.

In reply to the last question, as to how far down in the lungs the tubercle bacilli reach, I may say that the most frequent localization of these tubercles is subpleural, therefore it must be assumed that the bacilli penetrate to the terminal divisions of the respiratory passages.

TUBERCULOSIS IN RABBITS INDUCED BY DROPLET NUCLEI INFECTION: RESPONSE TO INITIAL INFECTION AND TO REINFECTION. H. L. Ratcliffe and (by invitation) W. F. Wells, Philadelphia, Pa.

Abstract. When rabbits were caused to inhale virulent bovine tubercle bacilli as separated cells in droplet nuclei, the initial tubercles developed at a highly uniform rate and followed a highly uniform pattern for a period of about 5 weeks after infection. Thereafter, progress of the disease varied with the animal and was proportional to the number of bacilli contained in the lesions. Hence it was concluded that rabbits do not differ in their inherent resistance to this organism, but differ widely in their ability to acquire resistance.

As judged by differences in the rate of development of initial tubercles after the fifth week, resistance developed slowly and the rate of its development varied widely. Experiments on inhaled reinfection show, however, that demonstrable levels of resistance were developed in all animals within 2 weeks after initial inhaled infection by small numbers of organisms. Within 5 weeks after small initial infection, resistance reached levels which inhibited reinfection by virulent bovine tubercle bacilli, inhaled as separated cells in droplet nuclei. In so far as could be determined by these experiments, the basic effect of acquired resistance of rabbits to this organism was the inhibition of its multiplication.

Discussion

(Dr. Max B. Lurie, Philadelphia, Pa.) I am glad to hear that this study on inhalation primary infection and reinfection confirms our work reported in a series of papers in *The Journal of Experimental Medicine* from 1928 to 1942. These have shown that in immunized animals the tubercle bacilli of reinfection are destroyed immediately if given in small numbers. If large numbers are employed the bacilli of reinfection fail to grow. We have also shown that the increased

destruction of the bacilli of reinfection is a primary function of the mononuclear phagocytes themselves. Humoral factors also play a rôle. You may remember that we placed mononuclears derived from normal and immunized animals, that had phagocytized the tubercle bacilli, *in vitro*, into the anterior chambers of the eyes of the same rabbits, and that we found that the cells derived from the normal animal permitted the free growth of tubercle bacilli, whereas the cells derived from immunized animals inhibited this growth, even though these cells had been transferred to a normal environment. Furthermore, this difference in the fate of the bacilli in normal and immunized animals has also been confirmed by Karl Jensen from Copenhagen, who also used inhalation methods, though his work was not as quantitative as that of Ratcliffe and Wells. He found that the chief difference between behavior of tubercle bacilli of reinfection as distinguished from bacilli of primary infection was that the former failed to multiply whereas the latter grew freely. I repeat that I am happy to learn that our work was again confirmed.

(Dr. Ratcliffe) In so far as we could determine from this material, the effect of the monocytes on the growth of the bacilli is not very striking. The most rapid points of growth of the bacilli seem to be outside of the cells, in the necrotic center of the tubercle, even when the tubercle is advanced, and in reinfection, if any growth takes place, it seems to take place outside of the monocytes, or outside of the other cells of the tubercle. It seems to me one might speculate as to the effect of antibodies rather than that of the cells themselves in this inhibition of the multiplication of the organisms.

PATHOGENICITY STUDIES OF TUBERCLE BACILLI, TYPE AVIUM, FROM A HUMAN INFECTION. William H. Feldman, Dorothy Hutchinson (by invitation), Virginia Schwarting (by invitation) and A. G. Karlson, Rochester, Minn., and Oak Terrace, Minn.

Abstract. Definitely proved progressive tuberculous infections in human beings are exceedingly rare. In this case report impressive evidence was obtained that supports the belief that a pulmonary infection in a 2-year-old child was due to tubercle bacilli of avian origin. The child, a member of a farm family, was ill with a pulmonary disease of undetermined etiology for several months prior to a diagnosis of *pulmonary* tuberculosis of the upper left lobe. On admission to the sanatorium a gastric lavage specimen was obtained which yielded a culture of acid-fast bacilli. The culture was unlike that of typical human tubercle bacilli and subsequently extensive studies of pathogenicity were done. The results indicate definitely that the organism studied is a typical avian tubercle bacillus. A survey of the child's environment before entering the sanatorium revealed several significant facts in support of the possibility of the farm animals being the source of the infection. The animals were tested with avian tuberculin with the following results: Among 430 chickens, 217 (50.4 per cent) reacted positively; among 6 swine, 2 reacted positively; and among 33 cattle, 10 reacted positively. None of the mammals reacted to mammalian tuberculin. Extensive lesions were present in most of the chickens examined at necropsy and cultures of avian tubercle bacilli were obtained from the birds examined. In addition the child frequently played with the chickens and often handled eggs. Tuberculosis was not present in the immediate family consisting of the parents and 4 other children, aged 6 months to 10 years. Under sanatorium care the child's condition has improved continuously. She will probably be released within the next few months.

Discussion

(Dr. Joseph D. Aronson, Philadelphia, Pa.) I should like to know whether the spleen was enlarged in this child, and whether blood cultures were made from this case. This is a thoroughly worked-up case and most interesting, because there is so much in the literature on avian tuberculosis in man which is questionable.

(Dr. Frank Dutra, Cincinnati, Ohio) In July last year 12 men who had been cleaning pigeon dung out of an attic became ill with what seemed to be an acute pneumonitis which required quite a long time to resolve. In December one of these men died of coronary thrombosis, and an autopsy was made. The lungs were grossly normal, but microscopically throughout the lungs there were a fair number of miliary lesions which strongly resembled tubercles. There were various stages, and many of them had gone on to complete fibrosis. Some similar lesions were found in the bronchopulmonary lymph nodes. The possibility that these men had been infected with avian tuberculosis was raised. Unfortunately, the body had been embalmed when the case came to my attention, so that no cultures could be made. Attempts were made by Dr. Albert Sabin to get some avian tuberculin from Dr. Feldman, and I believe tests are going to be made on the other 11 people who had been ill. We do know that there have been three other epidemics of similar pneumonitis in people working with pigeons in the United States since 1940, and I would like to know what Dr. Feldman thinks about the possibility of these cases being avian tuberculosis. Perhaps Dr. Feldman has some information as to whether avian tuberculin tests were done on the people in the other groups who were working with pigeons and developed pneumonitis.

(Dr. John R. Schenken, Omaha, Neb.) Were the infected chickens sensitive to human tuberculin?

(Dr. Stuart Mudd, Philadelphia, Pa., addressing Dr. Dutra) Did you have tests made on these pigeons for ornithosis?

(Dr. Dutra) Dr. Sabin did carry them out and they were negative. The possibility that the man who died might have had psittacosis also seems to be ruled out by the nature of the lesions.

(Dr. Feldman) I am sorry, Dr. Aronson, I cannot tell you about the possible enlargement of the spleen of this child. Dr. Hutchinson did not record that in her abstract. It is my impression that no attempts were made to culture tubercle bacilli from the blood.

As to whether pigeons might or might not be responsible for the respiratory infection in the human beings mentioned by Dr. Dutra, I think one guess is as good as another. I think we should first establish in those pigeons that remain whether they have tuberculosis. If they have not, this information will save a lot of subsequent work.

(Dr. Dutra) All that has been recovered from the pigeons is *Toxoplasma*.

(Dr. Feldman) As to whether tuberculous chickens react to human tuberculin, we have found that a very small percentage do react.

SEROLOGIC REACTIONS OF PATIENTS WITH SARCOIDOSIS TO ANTIGENS OF MYCOBACTERIUM TUBERCULOSIS. William H. Carnes and (by invitation) Sidney Raffel, Stanford University, Calif.

Abstract. The cause of sarcoidosis has not been established. Although it is rarely claimed that tubercle bacilli can be identified in relation to the lesions of the disease and the great majority of the patients fail to react to tuberculin,

a number of investigators adhere to the theory of tuberculous origin. The incidence of complement-fixing antibodies in the sera of 22 cases of sarcoidosis has been investigated with the use of a high molecular weight protein of the tubercle bacillus. More than half the cases were tested also with several other types of antigen including whole and defatted bacilli, protein, lipid, and carbohydrate preparations. The diagnosis was supported in each instance by biopsy. The patients were 19 to 65 years of age (mean age 28 years). Tuberculin tests were reported in 20 of the cases, 4 of which were positive to doses of 0.1 or 1.0 mg. Portions of the biopsy specimens of 16 cases were inoculated into guinea-pigs, all of which proved negative for tuberculosis. Altogether, 6 cases (27.3 per cent) gave positive serologic reactions with one or more antigens. No positive reactions were obtained with carbohydrate antigen in these cases or any of the controls. For comparison with the cases of sarcoidosis, a group of 26 cases of active tuberculosis, bacteriologically verified, was tested similarly. Altogether 16 of these (61.5 per cent) gave positive fixation reactions. Only one case of tuberculosis had a negative tuberculin test and this patient's serum had the highest titer of antibody (160 units per cc.) found in any serum. Sixty-seven per cent of the positive sera of tuberculous patients had titers of 40 units of antibody per cc. or higher with the protein antigens, whereas only 38 per cent of the positive sera of patients with sarcoidosis had titers of 40 units per cc., and none were higher. A further comparison was made with two groups of preclinical medical students recently tested with tuberculin. Of 30 students positive to the first test dose of PPD, but having no clinical or radiologic evidence of active tuberculosis, 10 (33.3 per cent) gave positive serologic reactions. None of a group of 29 students, negative to the second test dose of PPD, gave positive serologic reactions. These results lend no support to the theory that sarcoidosis is due to the tubercle bacillus. The incidence of positive serologic reactions to antigens of *Myco. tuberculosis* found in cases of sarcoidosis may reasonably be explained by previous unrelated tuberculous infection in a portion of the cases.

Discussion

(Dr. A. M. Pappenheimer, Boston, Mass.) We have studied a case in which tuberculosis had supervened on an initial sarcoidosis, and it was interesting to find in the lungs and elsewhere the two types of lesions coexistent, the typical sarcoid tubercles without caseation and without bacilli side by side with large caseating tuberculous lesions. It seems to me that this argues against the idea that sarcoidosis represents an altered reactivity of the tissues to the tubercle bacillus.

(Dr. Donald A. Nickerson, Salem, Mass.) Were skin tests done on these patients using as antigen emulsified material from the skin lesions?

(Dr. Joseph D. Aronson, Philadelphia, Pa.) It might be of interest to Dr. Carnes to know that we have vaccinated a number of cases of sarcoid intracutaneously with BCG vaccine. While these cases developed a local tubercle they still remained tuberculin negative. It would be interesting to have Dr. Carnes follow the cases serologically after injecting BCG to see what would happen.

(Dr. Wiley D. Forbus, Durham, N.C.) I hope that what I say will not be regarded as facetious in any respect, but may I raise a question? Would it not be a good idea for some ingenious man, and we must have lots of them, to attack the problem of sarcoidosis from a little different point of view? Instead of proceeding on the theory that it must or must not be tuberculosis, why not adopt the view that it may be any one of a number of other granulomatous

diseases about which we know little at the present time? It would seem to me that study of the problem of the possible relation of sarcoidosis to tuberculosis has now reached the stage that the study of the problem of the possible identity of Hodgkin's disease and tuberculosis long ago reached. Some ingenious young man might take another tack and help us along faster with the problem of the etiology of sarcoid. Certainly, Dr. Carnes has now exhausted one, and perhaps the last, of the possibilities of identifying the disease with tuberculosis.

(Dr. Carnes) I have also seen what Dr. Pappenheimer referred to, that is, the coexistence of typical caseous tuberculosis and sarcoidosis, and I think that my reaction is the same as his, namely, that it is further evidence that the sarcoid reaction is not on the basis of a peculiarity of the patient, as has been suggested, which would account for a different type of reaction to the same etiologic agent.

We have tried a good many patients with lymph nodes, not skin, Dr. Nickerson, testing them in a manner analogous to the Frei test. The emulsion was made in the way the old Frei antigen was made. Although there have been frequent positive reactions reported from the Scandinavian countries, we have not been able to confirm them, but we are still working on it, and have gotten some of the antigen from Dr. Danbolt in Oslo.

In view of Dr. Aronson's statement about the failure of patients with sarcoid who were inoculated with BCG to develop a positive tuberculin test, I think it would be very interesting to find what happens serologically to these patients in comparison with a group of nonsarcoid cases. We have not done this.

Dr. Forbus has pointed out that many etiologic agents will produce a lesion which is essentially like that of sarcoidosis, and that gives us a great deal of difficulty in determining whether there is still another unknown etiologic agent at the basis of the group of cases which we call sarcoidosis. The reasons are too numerous to try to be given right now, but a good many people still believe that there is an etiologic agent or a pathologic and clinical entity in this group of cases of sarcoidosis, in spite of the fact that there are a number of other agents which will simulate the pathologic lesion.

THE MICROSCOPIC STUDY OF LYMPHOID TISSUE IN THE PANCREAS AND ITS RELATION TO LYMPHOMATOSIS IN CHICKENS. Alfred M. Lucas and (by invitation) Eugene F. Oakberg, East Lansing, Mich.

Abstract. Not received.

THE PRODUCTION OF HEMOSIDEROSIS OF THE LIVER IN THE RAT BY DIETARY MEANS. T. D. Kinney, and (by invitation) D. M. Hegsted and C. L. Finch, Cleveland, Ohio, and Boston, Mass.

Abstract. Groups of adult rats were placed on 4 different diets. Control diet 1 consisted of purina dog chow alone, and control diet 2 was made up of purina dog chow plus 2 per cent ferric citrate. Diet 3 contained 80 per cent corn grits and 20 per cent lard. Diet 4 was made up of 98 per cent of diet 3 to which 2 per cent ferric citrate had been added. The animals were fed the diets *ad libitum* for periods of from 7 to 32 days and sacrificed. Both control groups did well. The animals on the corn grits and lard diet lost weight but, in the animals receiving this diet plus iron, weight loss was more precipitate and severe. The differences in the values for the iron content of the livers were striking. In group I they ranged between 7.3 to 13.24 mg. per 100 gm. of liver; group II, 8.5 to 15.6 mg.; group III, 5.4 to 30.1 mg.; and group IV, 35.5 to

93.6 mg. There was no significant variation in the serum iron levels between groups I, II, and III. This was true as well for determinations of the iron-binding capacity of the serum. However, there was a marked increase in the serum levels for the animals in group IV. At the same time the iron-binding capacity levels fell to zero in all but one animal.

Microscopic examination of the livers in group IV showed marked deposition of hemosiderin in the Kupffer cells and in the liver cells about the portal areas. Increased iron was present also in the bone marrow. This was not the case in any of the animals in the other 3 groups.

The mucosal block which has been postulated as the mechanism for controlling iron absorption in proportion to body needs has been overcome in the animals fed the corn grits diet.

Discussion

(Dr. Richard H. Follis, Jr., Baltimore, Md.) I should like to ask if control studies were made restricting the growth on purina with or without iron. I think it is well recognized that if you restrict the growth of animals by one means or another one can produce hemosiderosis of the spleen and liver. The explanation is probably that if you restrict the growth of the animal there is no necessity for as much iron to make hemoglobin as with the normal rate of growth. I would predict if you restrict the growth of the control animals you will probably get the same result in these as in your experimental animals.

(Dr. Russell L. Holman, New Orleans, La.) I should like to ask if Dr. Kinney has any direct data incriminating corn grits rather than some other ingredient of the diet, such as lard.

(Dr. Joseph J. Lulich, Madison, Wis.) Have you analyzed these organs to find out whether or not they contain ferritin?

(Dr. H. Edward MacMahon, Boston, Mass.) Was there any suggestion of early cirrhosis in any of the livers?

(Dr. Kinney) This paper really represents what we consider a preliminary report of a group of studies on the subject. Subsequent papers will report the work still under way.

We thought of the matter of growth as one of the possible causes of the deposition of hemosiderin, and we were also aware of Dr. Follis' paper on pyridoxine-deficient animals in which hemosiderin was deposited in the livers and spleens of these animals. As I remember Dr. Follis' paper, the animals were on the diets for at least 60 days or more. In our animals iron absorption takes place very rapidly as illustrated by the animal receiving corn grits diet and iron in which there was a marked elevation of the liver iron after 7 days. Further, in another group of experiments not reported today, serum iron levels were determined in fasting animals given single doses of iron mixed with the control and corn grits diets. When the iron was given with purina alone the serum iron showed only a slight initial rise, but when the iron was given mixed with the corn grits diet there was a slight initial rise followed by a prolonged rise. We felt then that these changes occurred too rapidly to be due to starvation alone.

The corn grits in the diet is not responsible, because animals fed rice or white bread instead of the corn grits show the same deposition of iron.

The livers, spleens, and other organs have not been analyzed for ferritin. There was no evidence of cirrhosis in the livers of these animals. In this connection it should be pointed out that the animals were on the diets for relatively short periods of time, usually no longer than 33 days, and although there was consider-

able fat visible in the livers no scarring was found. This is a severe diet and the animals do poorly and do not live much longer than 5 or 6 weeks. The diets were supplemented in various ways in an effort to determine the factor or factors responsible for the excessive absorption of iron. Casein was only slightly effective, while a complete salt mixture was more effective. The addition of phosphate salts to the diet greatly reduced, but did not completely prevent, the rise in the iron content of the liver.

HEMOGLOBINURIA (BLACKWATER FEVER) IN THE MONKEY WITH A CONSIDERATION OF THE DISEASE IN MAN.* R. H. Rigdon, Galveston, Texas.

Abstract. Hemoglobinuria occurred in 10 monkeys infected with *Plasmodium knowlesi*. The pathogenesis is discussed from the standpoint of an excessive amount of pigment in the blood stream which cannot be removed by the reticulo-endothelial system since the latter is already filled with malarial pigment. The excess of hemoglobin pigments, therefore, is filtered through the glomeruli. The tubular degeneration results from the acidosis that accompanies the disease and is not the result of the casts that form in the lumina of the tubules. The oliguria apparently may result from the shock which is the result of the disease rather than as the result of a mechanical blockage of the tubules by the precipitated hemoglobin.

Discussion

(Dr. Joseph F. McManus, Birmingham, Ala.) Have you estimated the non-protein nitrogen or blood urea in these animals?

(Dr. Joseph J. Lalich, Madison, Wis.) Have any blood pressure studies been done on these animals?

(Dr. Rigdon) We did not make any observations on these animals with regard to the retention of nonprotein nitrogen. There are other observations in the literature that would indicate that such does occur.

With regard to the blood pressure in these animals, we did not take it. This is only a part of a study in malaria in which we feel that severe anemia and anoxemia do lead to shock. We feel that these animals show evidence of shock because they respond so nicely to the transfusion of blood and the giving of oxygen. A low blood pressure may be the basis for the decrease in the amount of urine, or the anuria that may accompany blackwater fever. I think that decreased filtration rate is more important in the production of anuria than mechanical blockage of the collecting tubules by casts.

STUDIES ON THE PATHOGENESIS OF EXPERIMENTAL HEMOGLOBINURIC NEPHROSIS IN RABBITS WITH SPECIAL REFERENCE TO THE LATE MANIFESTATIONS.* Joseph J. Lalich (by invitation), Madison, Wis.

Abstract. Experimental hemoglobinuric nephrosis was consistently produced in rabbits by withholding water and food for 3 days prior to the intravenous injection of 1.8 gm. per kg. of homologous hemoglobin. In those rabbits which survived the acute phase of hemoglobinuric nephrosis, a nephrectomy was performed 13 to 17 days following the initial injection of hemoglobin. The rabbits were killed 34 to 116 days after the surgical operation, thus permitting microscopic examination of early and late manifestations of this disease in kidney sections of 12 rabbits.

In the surviving rabbits in the early phase, the total cast number varied from 4

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

to 188 in 10 low-power fields. Associated with the precipitation of hemoglobin there was tubular dilatation with flattening of the epithelial cells. There were also local areas of vacuolization of epithelial cells in the proximal convoluted tubules and in Henle's loops. During this period the rabbits manifested transient elevations of nonprotein nitrogen ranging from 50 to 250 mg. per cent. Following the nephrectomy the rabbits continued to eat and gain weight. Examination of kidney sections removed at autopsy demonstrated the transient nature of hemoglobinuric nephrosis when the animals do not die during the acute phase. In 7 rabbits no pigment casts were found in one longitudinal section. In 5 the total cast count varied from 1 to 5 in 10 low-power fields. Tubular dilatation and degeneration of epithelial cells was no longer evident.

After an animal survives the acute phase of hemoglobinuric nephrosis, death does not occur. Rabbits with extensive deposition of pigment, tubular dilatation, and epithelial degeneration are able to tolerate still further reduction of functioning renal tissue by nephrectomy.

Discussion

(Dr. Bernard Black-Schaffer, Durham, N.C.) I should like to ask whether kidney function tests were carried out at the time of nephrectomy in animals 5, 7, and 10, in order to obtain some idea of the functional potentialities of the one remaining kidney, which probably suffered the same severe changes as were demonstrated in the photographs of the excised kidneys.

(Dr. Virgil H. Moon, Philadelphia, Pa.) This presentation is in a field which is closely related to the one in which we have been deeply interested, that is, the changes occurring in the kidney, morphologically and functionally, incident to shock from various causes. There is disagreement among observers as to where these changes occur, whether they are limited to one portion of the nephron or whether they affect all portions of the nephron rather uniformly. These experiments are of similar type to those we have made, and probably the mechanism of effects is not very different. I should like to ask Dr. Lalich if in his examinations he noted the location of degenerative changes in one portion or another of the nephron.

(Dr. Lalich) The only functional studies done on these animals were plasma nonprotein nitrogen determinations, and, of course, under the conditions of this experiment practically all of the animals manifested some rise of nonprotein nitrogen, sometimes to as high as 260, with a subsequent return to normal levels. I should state that these operations were done during the period of declining nonprotein nitrogen. In most instances the nonprotein nitrogen at the time of surgery was slightly above normal levels, or within the normal range.

In answer to Dr. Moon's question, the majority of casts accumulated in the distal convoluted tubules, then proximal to that we found the dilatation, if it occurs. Dilatation does not necessarily have to occur. The degenerative changes which appear occur in the loops of Henle and the proximal convoluted tubules.

HYPERSPLENIC EXTRACTS. AN EVALUATION OF THEIR EFFECTS ON THE HEMATOPOIETIC ORGANS OF MICE. William R. Platt, Louisville, Ky.

Abstract. Acetone extracts of spleens removed from normal, thrombocytopenic, and neutropenic patients were prepared according to the original method of Troland and Lee. After the completion of control studies, intraperitoneal injections were made into 100 mice of three different strains (Swiss, A, and C57). Observations were then made on the peripheral blood, femoral marrow, lungs, liver, spleen, and kidneys. A discussion of similar experimental attempts by

other workers, an evaluation of the results obtained, and finally the possible clinical applications of such splenic extracts are related in detail in the complete report.

DISSEMINATED ARTERIOLAR AND CAPILLARY PLATELET THROMBOSES. Ira Gore (by invitation) and Nathan B. Friedman, Washington, D.C.

Abstract. Since Baehr, Klemperer, and Schiffrin described 4 cases of disseminated arteriolar and capillary platelet thrombosis in 1936, 15 cases have been reported in the literature. There has been considerable speculation regarding the basic mechanism involved. Although a few authors have postulated a primary endothelial lesion, such a premise has been minimized by a few and denied by others. Five cases of this disease were in the files of the Army Institute of Pathology and through the courtesy of Dr. Klemperer and Dr. Schlesinger, slides of 3 previously reported cases were made available for comparison. The findings in these cases indicated a definite vascular lesion of a type previously undescribed as the exciting cause of the syndrome. This early lesion is not often found, since the prompt action of the blood-clotting mechanism results in the formation of the fully developed lesion by the time of death. The latter has been described in the literature and its widely disseminated distribution in arterioles and capillaries gives the disease its name.

The vascular lesion to which the process is attributed consists of the accumulation of hyaline material beneath the endothelium of an occasional capillary or between the endothelium and muscularis of an arteriole. Neither the subendothelial reticulum nor the elastica of the arteriole is involved primarily. In fact, a splitting of the reticulum fibers is regarded as evidence of the formation of the lesion within the wall of the vessel. With the Schiff periodic acid staining reaction the hyaline material takes a bright red hue which contrasts sharply with the yellow color assumed by the remainder of the tissue. The process is a segmental one and many arterioles and capillaries do not exhibit it. In focal areas swelling occurs, evidently by imbibition from the plasma, to form a nodule which protrudes both into the lumen, carrying the overlying endothelium with it, and away from it, causing a defect in the vessel wall. Progressive swelling leads to rupture of the overlying endothelium and provides the focal injury responsible for the platelet thrombus. It seems reasonable to assume that swelling occurs rapidly, for otherwise the well known capacity of the endothelium to proliferate would prevent rupture. It is postulated that the lesion represents a defect of the intercellular endothelial cement substance which has recently been shown to be largely responsible for the permeability of capillaries.

In the earliest of the thrombotic lesions, a bimorphic character is evident. The peripheral portion is composed of the amorphous hyaline material originating from the lesion of the vessel wall; the luminal portion is granular and composed of aggregated platelets. Endothelial proliferative reaction which is initially absent, develops promptly from the intact endothelium at the margins of the initiating thrombus. There is a striking tendency for the thrombi to propagate along the length of the vessel and in many such instances the endothelial growth forms a sleeve-like process investing the thrombus. By contrast, the cells lining the lumen of the vessel containing the lesion are unaltered, a point which is indicative of the focal origin of the investing endothelium. The bulk of the thrombi seen ordinarily in the tissues consist of transections of such propagated thrombi.

The mode of organization provides further support for the primacy of the vascular injury. The endothelial proliferation is clearly an effort to re-establish the integrity of the vascular lining and is manifested first by growth over the

surface of the lesion. When this has been accomplished, there is secondary invasion of the thrombus. Organization does not occur from the base of the adherent thrombus as would be expected if the lining endothelium at that point were previously intact.

Discussion

(Dr. James F. Rinehart, San Francisco, Calif.) Is this a disease in itself, or are these cases of lupus? There is a great similarity, I believe, in certain of the lesions with those seen in disseminated lupus. Is this a separate picture, or is it related to disseminated lupus?

(Dr. Paul Klemperer, New York, N.Y.) I would like to answer Dr. Rinehart's question rather than to discuss the paper, although there is much to be said about it. I hope Dr. Gore will forgive me for replying to Dr. Rinehart. I think there is no question whatsoever of this peculiar condition and lupus being different. One of the points that might be deceptive is that in the original reports in 1925 and 1935 the patients were women, later on cases were reported in men. Except for the fact that there are vascular lesions in both diseases, there is no similarity.

(Dr. Bernard Black-Schaffer, Durham, N.C.) Siegmund described a lesion very similar which has been called in the German literature "Siegmund's nodes." He found them in cases of acute infectious disease. I wonder if the authors have compared this to their lesions.

(Dr. H. Edward MacMahon, Boston, Mass.) In the pictures of this disease that we have been shown today, there appear to be two separate and distinct vascular changes. In one of these the basement membrane of small vessels shows simply fusiform or nodular swelling; in the other, the endothelium is injured or lost and the intima shows a platelet and fibrin deposition. Were these supposed to show two stages of the same process or two distinctly different manifestations of the same disease?

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder whether fat stains were done on the early lesions. I think, upon comparing them with other findings, as in the Kimmelstiel-Wilson lesions of the glomeruli in diabetic patients, that they may be found positive.

(Dr. Louis Lichtenstein, New York, N.Y.) My question has to some extent been anticipated by that of Dr. Black-Schaffer. I would like to ask Dr. Gore whether there was anything distinctive about the clinical picture of the cases he presented.

(Dr. Gore) I would like to thank Dr. Klemperer for answering Dr. Rinehart's question, and I fully endorse his stand. I do not see any similarity between this condition and disseminated lupus.

In regard to Dr. Black-Schaffer's question, I am not familiar with Siegmund's article. I have not seen similar vascular changes in other conditions. This is the first time I have seen this particular lesion.

The question of Dr. MacMahon I believe is hard to answer morphologically, but Chambers, observing capillaries directly under controlled conditions, has shown that damage to the endothelial cement substance results in softening and there is a dropping off of material into the blood stream so that the lesion of the vessel wall, once the endothelium is ruptured, does not persist. Ordinarily, in tissue sections, too, we see cross sections of the propagated thrombus much more frequently than the focal point from which it arose.

In reply to the question concerning fat stains, we have not done any fat stains on the initial lesion. However, from their staining characteristics I would not suspect fat to be present.

Clinically, these patients have a characteristic clinical course featured by vague

and poorly defined prodromal symptoms, a rapidly progressive anemia, purpura, and thrombocytopenia. Invariably severe nonlocalizing neurologic manifestations occur before death. There does not appear to be a clinical common denominator as an etiologic or predisposing factor.

PLATELET THROMBOSIS IN HUMAN HEMOSTASIS: A HISTOLOGIC STUDY OF SKIN WOUNDS IN NORMAL AND IN PURPURIC INDIVIDUALS. Howard D. Zucker (by invitation), New York, N.Y.

Abstract. The histologic appearance of serial sections of skin puncture wounds, obtained for biopsy from 10 to 20 minutes after injury, was studied with the purpose of investigating the mechanism of hemostasis in normal and purpuric individuals. Hemorrhage from severed muscular vessels up to $250\ \mu$ in diameter is normally arrested by platelet thrombi formed at the site of vascular injury. Platelet thrombi do not form in severed true capillaries. Fibrin is normally seen within the wound tract; it is not seen in the lumen of cut vessels. In idiopathic thrombocytopenic purpura, platelet thrombosis is never seen. Fibrin formation may occur in wounds in idiopathic purpura; its appearance may be delayed for at least 15 minutes. Without platelet thrombosis, fibrin formation within the wound is inadequate to arrest hemorrhage from muscular vessels within 3 minutes. The similarity of the histologic appearance of human puncture wounds to that described after experimental vascular injury in other mammals suggests considerable similarity in mammalian hemostatic mechanisms.

Discussion

(Dr. Joseph Tannenberg, Batavia, N.Y.) I am very much interested in this paper, since I conducted experiments between 1925 and 1930 on animals in which the walls of the small arteries and capillaries could be made visible and microscopically studied during the time of hemostasis. In these experiments I found that the segmentary contraction of the small arterioles and what we called with Ricker and others stasis in the capillaries, *i.e.*, a conglutination of the erythrocytes, were the most important factors in bringing about stoppage of the bleeding. In addition to this the pressure on the veins of the hemorrhage into the tissue seemed to be an important factor. A developing thrombosis and fibrin formation in the bleeding wound, and particularly in the torn wall of small veins, were considered major contributory factors of hemostasis. The last factors may be absent in individuals with thrombopenia and purpura and account for the modification of hemostasis.

(Dr. Zucker) I am familiar with Dr. Tannenberg's papers. In so far as contraction of the arterioles or of any of the muscular vessels is concerned, I am sure that it is an important factor in hemostasis. On the basis of what I have seen in my sections, I would lay primary stress, if anywhere, on the platelets, but certainly the muscular factors are exceedingly important. As far as the conglutination of the red blood cells is concerned, that is something which has not been seen in any of my controls nor in any purpuric sections, and I cannot make further comment on it.

THE INCIDENCE OF RHEUMATIC HEART DISEASE IN INDIVIDUALS WITH CONGENITAL MALFORMATIONS OF THE HEART. S. H. Durlacher and (by invitation) Emil C. Beyer, Edgewood, Md., and New York, N.Y.

Abstract. Consecutive autopsies to the number of 7925, performed in the Department of Pathology, Yale University School of Medicine, were analyzed. There were 6,853 cases over 2 years of age, the age of the youngest rheumatic indi-

vidual. Five hundred and nine hearts showed anatomic lesions of rheumatic heart disease and of these, 43 had active rheumatic myocarditis at the time of death. Among 55 cases with congenital malformations of the heart there were 22 with rheumatic stigmata, and of these, 4 had active rheumatic myocarditis at the time of death. The rheumatic rate among cases with congenital malformations is statistically significantly higher than in the group without congenital lesions even when the rheumatic rate in the latter group is corrected for the age distribution of the former. Rheumatic lesions occurred in 4 of 6 instances of interauricular septal defect, in 2 of 4 instances of tetralogy of Fallot, in 2 of 4 cases of interventricular septal defect, in 1 of 3 cases of persistent truncus arteriosus, in 2 of 6 cases of patent ductus arteriosus, and in 11 of 29 instances of minor malformations. Active rheumatic myocarditis occurred in 2 instances of interventricular defect, 1 case of tetralogy of Fallot, and 1 case with bicuspid pulmonic valves.

Discussion

(Dr. Jesse E. Edwards, Rochester, Minn.) This presentation comes with some surprise to me in that it has been my personal experience that rheumatic heart disease is not unduly associated with congenital malformations of the heart. On the other hand, when one examines hearts from cases of congenital malformations it has been rather common to find that there are localized areas in the endocardium in which there is fibrosis. The fibrosis may be diffuse in one chamber, or it may be localized, and there may be some thickening of the valve leaflets. Most of these endocardial reactions, I believe, are secondary to mechanical alterations on the basis of the congenital malformation. For example, in patent ductus arteriosus it is common, almost without exception, to find the endocardium on the left side of the heart, particularly, showing considerable fibrous thickening with collagen and elastic tissue. My reaction is that the dilatation of the chambers of the left side results in the production of connective tissue in the endocardium, a production which is on the basis of a mechanical rather than an inflammatory change. The same holds true of a defect of the atrial septum. One frequently finds that the leaflets of the mitral valve are thickened, but when one examines those valves carefully, one usually does not find changes in the chordae; they are not fused, shortened, or thickened, and again I believe that dilatation of the atrium, which will cause stretching of the valvular tissue, results in the production of fibrous tissue. In defects of the interventricular septum there may exist localized endocardial thickening which probably can be explained on the basis of mechanical trauma by eddies of blood. It is my opinion, therefore, that in evaluation of cases of congenital heart disease, considerable caution should be exercised before one says rheumatic fever is present, since mechanical factors alone may account for certain endocardial changes. Microscopic evidence of rheumatic carditis is, of course, conclusive.

(Dr. William H. Carnes, Baltimore, Md.) Did you find very many instances of bland vegetations on the valve leaflets, particularly on the right side of the heart with pulmonic stenosis, such as I have encountered, and find frequently commented on in reports of these cases? Were the vegetations of this type which you saw on the valves regarded as being rheumatic vegetations?

(Dr. Alfred Angrist, Jamaica, N.Y.) What percentage of the hearts showed active rheumatic lesions in the myocardium, and did one see active rheumatic verrucae? My own experience parallels that of the discussers, that the association is uncommon. The fact that some of the localized lesions on the valve have mimicked the findings in rheumatic distortion may well represent congenital defects of the valve structures, associated with congenital deformity, because often in newborn congenital hearts you see similar gross distortions of the valve.

(Dr. Durlacher) I agree with many of the things Dr. Edwards said. I was surprised in this analysis to find the high incidence of rheumatic heart disease among patients with congenital heart disease over the age of 2 because I did not expect it. We had a peculiar coincidence when we began operating on cases of tetralogy of Fallot, and one patient died with an acute rheumatic myocarditis. That was the start of the analysis. I was very cautious about interpreting the results when scars were the only criterion for the diagnosis of rheumatic heart disease, and for that reason I separated what I called the active and the inactive cases and subjected them to separate statistical analysis. I do not know that perivascular scarring is any more reliable as a diagnostic criterion for rheumatic heart disease than endocardial scars and neither are reliable for diagnosis. The value of the interpretation of these depends on the ability of the men performing the autopsy. For this reason I tried to get away from that objection by taking only the major valvular deformities, such as were shown here, as criteria, and only cases with such deformities were included. The analysis of the acute cases leaves, I think, no question. I grant the number is small, only 4, but there were 4 of 55 cases of congenital cardiac disease, an incidence of 7 per cent, which is statistically higher than the rheumatic rate in the noncongenital group.

Bland vegetations were encountered frequently in many of the cases, but I was well aware that thrombi on the valves and mural endocardium are quite common in congenital malformations, and they were not used as criteria for rheumatic heart disease.

In answer to the question about the number of active cases, we had 7 per cent active rheumatic carditis of 55 cases of congenital heart disease living over the age of 2.

The question of valvular deformities found in newborns did not enter this series as the analysis included only cases over the age of 2 years and these newborn deformities fell out.

UNEXPECTED DEATH IN CHILDREN WITH RHEUMATIC HEART DISEASE. T. R. Hamilton, and (by invitation) W. A. Tanner, E. M. Pebley, Kansas City, Kansas, and G. S. Voorhees, Leavenworth, Kansas.

Abstract. This report cites 4 instances of unexpected death in children in whom rheumatic heart disease was the pathologic diagnosis. The ages at the time of death were 15, 11, 4, and 2½ years in this group of 2 boys and 2 girls, of whom 2 were white and 2 Negro.

The most dramatic instance was that of a 15-year-old white boy who dropped dead at the blackboard at school. A sclerosing type of lesion involving smaller branches of the coronary arteries was rather marked in this case. There was a history of four rheumatic episodes over a period of 7½ years under medical management. The second case was that of an 11-year-old colored girl found dead in bed on the fourth day of hospitalization. This was shortly after she complained of pain in the chest along with nausea and abdominal distress. She had had her initial rheumatic episode only 4 months before admission and had experienced precordial pain during the last 3 months. Adhesive pericarditis was marked in both of these cases with synechia cordis in the latter instance. In that case a narrow ostium of the left coronary artery was noted. Pancarditis was marked in these enlarged hearts which weighed 660 and 380 gm. respectively. The valvular lesions were not stenosing.

The two younger children were not known to have had rheumatic fever clinically. A white girl, age 2 years and 8 months, died suddenly during morning

care on the first day of hospitalization. This occurred shortly after she had complained of pain in the chest. The fourth child, a 4-year-old colored boy, suddenly collapsed while at play and died in the emergency room of the hospital. Pathologic consideration of these last 2 cases centered on cardiac enlargement. In the 4-year-old child the heart weighed 150 gm., which is three times normal size, but in the 2-year-old child there was no accurate weight. Anitschkow myocytes appeared most strikingly proliferative in vascular areas, particularly along a delicate verrucous margin of valvular endocardium in the youngest child. Pneumonitis was a feature in these 2 cases.

The most striking coronary lesion was noted in the case of the oldest boy; however, he had had fairly persistent cardiac arrhythmia with auricular fibrillation. A narrow coronary ostium appeared to be significant in the case of the older girl. In 3 of the cases pericarditis was particularly striking. Myocardial involvement was a prominent feature common to all.

Discussion

(Dr. Jacob Werne, Jamaica, N.Y.) Most of the pathological conditions which are responsible for death may be associated at one time or another with sudden death. There are certain conditions associated with sudden death which are frequently overlooked unless specific search is made. I refer here to the frequency with which upper respiratory infections are responsible for death in early life. For the discovery of these conditions at autopsy, it is important that the neck organs, mastoids, and middle ears be examined particularly. The presence of lesions in these areas may often explain why subjects with other conditions such as rheumatic heart disease die sooner and more suddenly than might otherwise be expected. With regard to the patient dying 10 days after vaccination, the possibility of post-vaccinal encephalitis must be considered.

(Dr. Arthur C. Allen, New York, N.Y.) I should like to mention that Aschoff bodies of rheumatic myocarditis have been found in a number of instances at the Army Institute of Pathology in previously healthy people who died sudden traumatic deaths. This fact does not negate Dr. Hamilton's findings, but it does introduce a statistical nicety.

(Dr. Virgil H. Moon, Philadelphia, Pa.) Many of those who die of unexplained causes, or of inadequate causes, have been found to have hypoplasia of the adrenal cortex and have lymphoid hyperplasia associated with that. I should like to ask the authors whether examination for these features has been made.

(Dr. Hamilton) The neck organs were not commented upon in all cases. In the last 2 cases pneumonitis was a feature, and it was interpreted as of rheumatic type. There was some evidence of decompensation in the second case, that of the girl who died after angina of 3 months' duration. She had chronic passive congestion of the liver and some congestion of the lungs.

In regard to Dr. Werne's question about the possibility of lesions in the brain following vaccination in case 4, it is unfortunate that examination of the brain was not allowed. There was no evidence of embolism in these cases, which is important, particularly because in the series of Stroud and Twaddle on 15 years' observation of rheumatic heart disease in children, of the 9 with sudden death 5 were under 16 years old, and there was embolism in the majority of these cases. We did not observe any indications of embolic phenomena in our cases.

In regard to Dr. Allen's comment, I think that it is of major interest in sudden death that Dr. Moritz, at the Army Institute of Pathology, analyzed 40,000 cases and found 5 in which there was evidence of rheumatic heart disease. That was an older group, and I did not mention it in this brief report for that reason.

In reply to Dr. Moon's question, we did not observe lymphoid hyperplasia or adrenal hypoplasia in this series.

THE FATE OF BLOOD INJECTED INTO THE ARTERIAL MEDIA. William B. Wartman, Chicago, Ill.

Abstract. These experiments, which have for their purpose the study of the fate of blood injected into the arterial media, were undertaken in order to determine whether or not hemorrhage into the media of an artery plays a part in the initiation of arteriosclerosis or of dissecting aneurysm. Eight dogs were used and a total of 26 hematomas was produced in 17 arteries. Blood from the same animal was injected into the wall of the common carotid and femoral arteries and abdominal aorta. Because of the extreme thinness of the normal canine intima it was found to be impossible to inject blood into it so that only medial hematomas were produced. The results were the same in all vessels and no difference was noted between venous and arterial blood. Study of hematomas at various ages from 3 to 352 days showed that the blood was removed from the media by gradual destruction of red blood cells accompanied by liberation of pigment and mild inflammation. Medial necrosis was present during the first 8 days, but was not observed after this time. The red blood cells disappeared between the 15th and 24th days. Hemosiderin pigment was observed first after 8 days and in most instances persisted for as long as 61 days, but in 2 cases it was found at the end of 392 days. Medial scars were first seen at the end of 48 days. They were composed of collagenous connective tissue and contained capillaries which usually disappeared between 61 and 169 days, although in 2 cases they were observed after 392 days. Moderate fibrosis and hyalinization of the intima occurred occasionally. In no case was there atheroma formation, intimal arteriosclerosis, or aneurysm formation, and no hemorrhage occurred from the new capillaries which grew in the hematomas. The whole process appeared to be one of organization of the hematomas, resulting in the medial scar or in the restitution of the arterial wall. The lesion was apparently self-limited and did not progress either to arteriosclerosis or dissecting aneurysm.

Discussion

(Dr. S. H. Durlacher, Edgewood, Md.) I find it interesting that there were no phagocytes filled with lipids in the media of these vessels. The injection of blood into the peritoneal cavity is followed by the accumulation of large phagocytes which stain with sudan III. I wonder if any of these were found.

(Dr. Wartman) We did occasionally find foreign body giant cells, but no phagocytes containing lipids.

PATHOLOGY OF AMINO ACID DEFICIENCY IN RATS. I. PHENYLALANINE DEFICIENCY.

R. L. Ferguson and (by invitation) Charles Schwartz, Vermillion, S.D.

Abstract. Weanling male rats were fed a completely synthetic diet for periods up to 28 days. Rats of corresponding ages were fed the same diet from which the essential amino acid, phenylalanine, was omitted. Control groups were carried along on a similar diet in which the amino acid mixture was replaced by a corresponding amount of vitamin-free casein, and on a diet in which the amino acid mixture was replaced by $2\frac{1}{2}$ times its weight of vitamin-free casein. All rats were weighed periodically and a record of food consumption was kept.

At intervals rats from each group were sacrificed and sections of all tissues and organs were taken for complete histologic study. The results to date indicate that in those rats fed the diet deficient in phenylalanine, there was a marked retardation of spermatogenesis.

COLITIS IN THE FOLIC ACID-DEFICIENT MONKEY WITH NOTES ON SIMILARITIES TO ULCERATIVE COLITIS IN MAN. James F. Rinehart and (by invitation) Louis D. Greenberg, San Francisco, Calif.

Abstract. Progress in nutritional research has been rapid. One of the most important advances was the development of a synthetic diet adequate for evaluation of a single deficiency in the rhesus monkey. With the conviction that the nutritional requirements of this primate would most closely approximate those of man, we have undertaken systematic studies of deficiency states in this species.

This report is concerned with our observations on folic acid deficiency. In the rather extensive literature on folic acid deficiency there is a surprising lack of detailed study of pathologic changes. Most of the experiments have been concerned with its influence on blood formation. When folic acid is removed from the diet, the animals ordinarily gain weight and remain active for 2 or 3 weeks. At this time they begin to lose weight. Usually in 4 to 5 weeks they develop diarrhea and become less active. Ulceration at the gum margin may develop and the weakness and the diarrhea become progressively more severe.

Our studies on the blood are in essential agreement with those previously reported. The animals develop moderate anemia and leukopenia. Administration of folic acid causes a reticulocyte response, rise in the white blood cell count, and improvement in the gums, bowel function, and general condition. During deficiency the polymorphonuclear leukocytes show characteristic degenerative changes. They become larger, the neutrophilic granules disappear, and bluish bodies are seen in the cytoplasm which is often vacuolated.

We have previously called attention to the characteristic and usually severe lesions which develop in the colon. All of the animals developed diarrhea and of the 7 animals examined post-mortem, 6 showed a structural colitis. In the earlier phases small irregular ulcers with purulent exudate appear in the congested and edematous mucosa. In time the lesions become more extensive. Cystic dilatation is seen in mucosal glands which are lined by atrophic and degenerating epithelial cells. Normal mucous secretory activity appears to be reduced and many of the cells show hyperchromatic nuclei. By others, colitis in folic acid deficiency has been ascribed to a concurrent bacillary dysentery developing in a weakened animal. By stool cultures on 6 of our animals, the Flexner dysentery bacillus was isolated from 2. The others failed to show pathogenic organisms. Our evidence indicates that folic acid deficiency specifically lowers the resistance of the colon to invasion by pathogens and that an ulcerative colitis may develop as a part of the deficiency disease even in the absence of pathogenic organisms. The lesions bear a very close resemblance to those of ulcerative colitis as it is encountered in man. It is of particular interest that one animal which we maintained in a state of chronic deficiency for 23 months showed practically complete loss of mucosa and a rigid, thick-walled colon with a narrow lumen corresponding to the lesion seen in the late stages of ulcerative colitis.

Our observations suggest that folic acid deficiency may be a factor in the pathogenesis of ulcerative colitis in man. This possibility is certainly deserving of critical clinical study.

Discussion

(Dr. John H. Fisher, London, Ont.) I should like to ask Dr. Rinehart if he saw any evidence in his animals of cirrhosis of the liver. We have recently seen a human case of long-standing, but relatively mild, chronic ulcerative colitis, in which an advanced cirrhosis of the liver was present.

(Dr. Russell L. Holman, New Orleans, La.) I should like to ask two questions.

First, have you any data on therapeutic tests, and second, have you observed any changes in other mucosae, for example, that of the respiratory tract? If you have not noticed any difference in the mucosa in other parts of the body, do you have any idea why it is present only in the colon?

(Dr. Jacob Werne, Jamaica, N.Y.) I wonder whether the sera of these animals from which no enteric pathogens were cultured were examined for antibodies.

(Dr. Rinehart) This group of animals did not have cirrhotic change in the liver. Some of the animals subjected to pyridoxine deficiency which we have studied have shown such changes. In respect to therapeutic trials, these we have not made.

With regard to other mucosal changes: we have found none, other than that described in the oral mucous membrane. I am unable to understand the pathogenesis of the lesion in the colon at this time.

I appreciate the suggestion of examination of the serum for antibodies to dysentery bacilli and will include it if further study is made. Our study was not directed toward colitis; initially it was simply a study of folic acid deficiency. I might point out one thing I neglected to say, that other animals subjected to equal degrees of inanition have not shown colitis, with one exception. A pyridoxine-deficient animal did develop an ulcerative lesion in the colon.

PATHOGENESIS OF CRYPTOCOCCIC (TORULA) MENINGITIS. Kornel Terplan, Buffalo, N.Y.

Abstract. In the majority of the post-mortem reports on cryptococcic infection recorded in the literature, the central nervous system was the only organ involved. All of the recorded, so-called pulmonary cases (about 20 altogether) were, possibly with a rare exception, associated with the well known lesions in the central nervous system, especially in their coverings. In most of the cases of *Torula* meningitis, including the generalized types with lesions in various organs and with miliary changes in the lungs, no morphologic data are available which could point to the respiratory tract as the portal of entry.

The anatomic and histologic findings of an otherwise typical case of cryptococcic meningo-encephalitis observed in a 28-year-old Indian laborer, who was admitted to a tuberculosis sanatorium for suspected tuberculous meningitis, are presented. Spinal fluid samples yielded abundant masses of *Cryptococcus neoformans*, the pathogenicity of which was established by animal inoculation. Death occurred 5 months after the initial symptoms of meningitis. Apart from the characteristic findings of gelatinous meningitis with small cysts in the cortex and wart-like granulomas in the dura, extending into the left gasserian ganglion, a well encapsulated, bean-sized nodule, found in a subpleural position in the right lower lobe, measured 11 by 6 mm. It resembled in consistency and color a typical primary caseated tuberculous focus. Histologic analysis revealed a well encapsulated area, including within the alveolar framework colony-like masses of yeast cells with well preserved mucinous capsules. The histologic structure of the tissue surrounding this focus pointed to simple capsule formation by collagenous fibers blending with the thickened pleura and containing numerous capillary vessels, moderate infiltrations of lymphocytes, and an occasional giant cell of the foreign body type. Part of the adjacent lung tissue was atelectatic. There were no other lesions produced by this fungus, either in the lung and its regional lymph nodes, or in any other organ, including the mediastinal pleura. The paranasal sinuses and middle ears were uninvolved. This primary tubercle-like lesion caused by the yeast cells was the only one which could have served as the source for the secondary hematogenous spread to the leptomeninges.

Discussion

(Dr. Wiley D. Forbus, Durham, N.C.) I should like to add a word in support of what Dr. Terplan has said about the primary pulmonary origin of the infection in cryptococcic meningitis. We have recently had an experience which helped us a good deal in this connection. In our case we found a primary lesion comparable in every respect to that which you have seen on the screen. The primary lesion lay immediately beneath the pleura. It had ulcerated through the pleura and spilled its contents into the pleural space, resulting in an extensive invasion of the pleural lymphatics and those of the parenchyma of the lung. This was followed by widespread dissemination, but there was no involvement of the meninges. After that experience we made a study of all of our other cases of cryptococcosis, including those in which no lesion had been found anywhere except in the meninges. We returned to the gross material, made careful serial sections of the lungs, and found the primary focus in every case as an old fibrous or caseous nodule. This observation, of course, does not rule out certain other possible primary foci. Those who are familiar with the problem will recall the paper published by Urbach and Zach in which there was extensive cryptococcic infection of the mouth. Obviously, such a lesion might serve as the point from which the organisms were disseminated to the internal organs.

(Dr. Terplan) I would like to add that a few rare observations have been recorded with unusual portals of entry of the cryptococcic infection. The case to which Dr. Forbus referred is apparently one of these; and there were a few others, one with a deep ulcer in the tongue, one with a lesion in the skin (from a razor blade), and also one where the rectum was thought to be the site of the original infection. The fact, however, remains, that in the majority of the cases reported in the literature only meningeal and cerebral involvement has been observed and the lesion or lesions in the lung were not detected.

THE PATHOLOGY OF HERPES SIMPLEX ENCEPHALITIS IN MAN, WITH A REPORT OF THREE CASES. Webb Haymaker, Washington, D.C.

Abstract. In 3 proved cases of herpes simplex encephalitis, previously reported in abbreviated form, the pathologic changes were very similar. The most striking lesions were in the cerebral cortex: they consisted mainly of massive necrosis, replacement of superficial lamina by large mononuclear and gitter cells, and perivascular invasion of parenchyma by mononuclear cells; in 2 of the cases there were myriad neuronophagic nodules. Topographic study on the available material disclosed that lesions were most severe in the cerebral cortex, hippocampal formation, subcortical white matter, claustrum and some of the basal olfactory nuclei, and affected to much less degree were the striatum, thalamus, and cerebellum. The pallidum, amygdala and spinal cord were spared. Type "A" intranuclear inclusion bodies were found in profusion in ganglion cells and oligodendroglia of the cortex and subcortical white matter, and to a lesser degree elsewhere. They stained most satisfactorily with hematoxylin and eosin.

Discussion

(Dr. Margaret G. Smith, St. Louis, Mo.) I was very much interested in Dr. Haymaker's presentation. The histologic lesions are much like those which we have seen in the one case which has been reported and in two others which have not been reported. I think there are several points of interest about these cases. All of the patients have been quite young people, one of ours being a month-old infant, the second a 14-year-old boy, and the third a 17-year-old girl. The possible route of entry of this virus into the central nervous system has

interested me, and also what relation there may be between the development of encephalitis and the individual's state of immunity. The last two of the cases that we have studied have been very interesting in that the lesions were confined almost entirely to the olfactory areas of the brain, suggesting that the olfactory pathway had been the route of entry of the virus into the central nervous system. In the case of the young infant we could obtain no serum from the mother to test for antibody for the herpes virus. The other two patients, as far as we could learn from their families, had never had herpetic skin lesions.

(Dr. Haymaker) As regards the occurrence of herpetic lesions over the preceding years in our cases, there is no history of such available.

The basal olfactory localization of the lesions referred to by Dr. Smith is of considerable interest, inasmuch as in one of our cases the initial symptom was that of "peculiar smells"; these persisted for 3 or 4 days, and then the patient complained of experiencing "peculiar tastes." Since olfactory sensation may be represented, in part at least, in the pyriform area, and since this region, together with the anterior perforated substance and nucleus accumbens septi, were markedly affected in our case, the olfactory pathways may well have been the route by which the virus gained access to the brain. Considered from the standpoint of the severity of the lesions, it could well be argued that the primary site of attack was the cerebral cortex.

One other point is worthy of comment, and that is that various stains were employed in an effort to determine which was the most satisfactory in demonstrating the inclusion bodies, and best results were achieved with the standard hematoxylin and eosin method.

TRANSFER OF IMMUNITY TO THE VIRUS OF ST. LOUIS ENCEPHALITIS TO SUCKLING MICE THROUGH THE MILK, DEMONSTRATED BY FOSTER NURSING. Margaret G. Smith and (by invitation) Betty B. Geren, St. Louis, Mo.

Abstract. Four groups of suckling mice 7 to 12 days of age, a total of 217, were used in this experiment. Two groups were mice born of mothers immunized by two intraperitoneal inoculations of the St. Louis encephalitis virus. The second intraperitoneal inoculation had been given 3 weeks prior to mating. The other two groups were mice born of non-immunized mothers. One group of mice born of immunized mothers was transferred at birth to non-immunized mothers for foster nursing. Similarly, a second group of mice born of non-immunized mothers was transferred at birth to immunized mothers. The two other groups of mice, one born of immunized and one of non-immunized mothers, were allowed to remain with their own mothers. The suckling mice were tested for resistance by intraperitoneal inoculations of the virus. The two groups of mice nursed by immunized mothers showed significantly greater resistance to the virus than did the two groups nursed by non-immunized mothers. The latter two groups showed no significant difference in resistance to the virus, although one group was born of immunized mothers and the other group of non-immunized mothers.

MYOCARDIAL LESIONS IN POLIOMYELITIS. Vera B. Dolgopel and (by invitation) Mary D. Cragan, New York, N.Y.

Abstract. Acute myocarditis has been found at necropsy in 16 of 87 cases of poliomyelitis. The ages of patients ranged from 13 months to 37 years. The foci of myocarditis were small, occupying only a small portion of a low-power microscopic field. Several lesions were usually found in the same section, but in multiple blocks from a heart lesions were rarely found in more than 2 blocks. The

posterior wall of the left ventricle and the posterior papillary muscle were the sites most frequently affected.

Lesions of three types were encountered. In the first type, which at low power showed only increase in the number of nuclei, individual muscle fibers were thin and cloudy, with long, wavy nuclei clinging to them. In the second, cellular collections, predominantly mononuclear, were present between myocardial fibers which usually were intact. The third type consisted of collections of cells in the interstitial tissue around the blood vessels. The cells usually were histiocytic mononuclear cells, but in one case the infiltrate consisted of polymorphonuclear leukocytes and lymphocytes.

Pneumonia was present in 4 cases; 3 cases showed minimal inflammatory lesions in the lungs, 9 showed no pulmonary inflammation. In 4 cases death was apparently caused by cardiac failure.

The incidence of myocarditis was 18.4 per cent in the entire material. However, in cases with one section available for examination the incidence was 9.3 per cent, and in 44 cases with multiple slides it was 27.2 per cent. The latter figure is probably closer to the actual incidence of myocarditis in fatal poliomyelitis.

CARDIOVASCULAR LESIONS IN ACUTE POLIOMYELITIS. Ted E. Ludden (by invitation) and Jesse E. Edwards, Rochester, Minn.

Abstract. By means of the method of Gross, Antopol, and Sacks for histologic examination of the heart, myocarditis was demonstrated in 14 (40 per cent) of 35 cases in which acute poliomyelitis was fatal. Myocarditis was classified as severe in 6 cases, moderately severe in 4, minimal in 3, and healed in 1. In those hearts classified as demonstrating severe myocarditis, focal coagulation necrosis of myocardial fibers and associated infiltration of neutrophils were conspicuous manifestations. The principal histologic evidences of myocarditis in the other specimens were perivascular collections of large mononuclear cells and minimal alterations of myocardial fibers. In the heart classified as "healed" there were focal lesions characterized by the complete disappearance of myocardial fibers; in these areas only myocardial stroma remained.

Three patients who had myocarditis presented distinctly unusual findings. In one there was rupture of the myocardium of the right atrium. In another there was acute vegetative endocarditis of the mitral valve, and in a third there was acute vegetative endarteritis of a patent ductus arteriosus.

Since animal inoculations were not carried out in this study, the relationship of the poliomyelitis virus to the observed lesions was not definitely determined. However, the high incidence of myocarditis among this group of patients and the frequency of myocarditis reported by Saphir and others would seem to indicate that in the presence of poliomyelitis myocarditis is actually due to the virus of poliomyelitis.

*Discussion of Papers by Drs. Dolgopol and Cragan, and
Ludden and Edwards*

(Dr. Jacob Werne, Jamaica, N.Y.) I believe it should be emphasized that these lesions are not at all specific. Similar lesions were described some time ago by Parker and Nye as tissue reaction to streptococcal infection. We have encountered these lesions frequently in association with fulminating infections. Their incidence in subjects dying with acute infection of whatever cause will vary with the extent of the histologic study.

(Dr. Kornel L. Terplan, Buffalo, N.Y.) In the post-mortem material of the large epidemic of poliomyelitis in 1944 in Buffalo, in the cases examined (about 40

of 60), only few minimal interstitial infiltrations of focal character were found in sections taken from the heart. In one case they were somewhat more marked. In about 15 cases the heart was not examined. Usually one section was taken from the right, and one from the left ventricle. The fatal cases in that epidemic were practically all of the bulbar type. In all, acute inflammatory conditions of the respiratory tract were observed, and it appeared to me that these lesions pointed to a failure of peripheral respiration as the immediate cause of death. In the pathogenetic consideration of the lesions found in the heart muscle, the inflammatory changes found in the lungs and in the upper respiratory tract should be considered as a possible factor.

(Dr. Dolgopol) I think I will agree with Dr. Werne that the lesions are probably not characteristic of poliomyelitis alone, and that this is one of those myocarditides observed in many infectious diseases.

As to pulmonary infections being the cause of myocarditis in our cases, 9 of the 16 cases showed no inflammation in the lungs or bronchi. We had a number of blocks from the lungs available for examination, and there was no inflammation in these 9 cases. There was pneumonia in 4, and only minor evidences of polymorphonuclear infiltration in a few alveoli in 3 others, so I would say that the majority of our cases were free of pulmonary infection.

(Dr. Edwards) Pneumonia was not a common factor in our cases. Of the 14 patients with myocarditis, 3 had pneumonia.

As to the implication that this lesion is caused by a virus, let me say that it is difficult to determine whether a virus is a direct infecting agent in a lesion of this kind. Even if a virus were isolated in the myocardium, it might be difficult to ascribe lesions directly to the virus. In poliomyelitis, myocarditis is something with which one must deal and I would like to say in reference to the recent study of Parker and his associates of cases in which the influenza virus was identified in the body, that they found myocardial lesions which were identical to those which we found in these cases of poliomyelitis.

OBSERVATIONS WITH IMPROVED ELECTRON MICROSCOPIC TECHNICS ON THE INTERNAL STRUCTURE OF *ESCHERICHIA COLI* CELLS AND THE GENERATION OF COLIPHAGE. James Hillier (by invitation), Stuart Mudd and (by invitation) Andrew G. Smith, Philadelphia, Pa.

Abstract. New lenses, objective apertures, and preparative technics have made it possible to begin investigation of the internal structure of bacterial cells. In electron pictures of normal *Escherichia coli*, strain B, the fine structure of the protoplasm is resolved as particles spaced in three dimensions; linear aggregation is apparent in thinner regions of the protoplasm. Adjacent to the ends of the cells and to the planes of division the protoplasm is relatively dense. Between these dense areas appear regions of low density containing granules and rodlets of very dense matter; these correspond in position and appearance with the chromatinic bodies described by C. F. Robinow.

Coliphage T₂ may cause lysis of *Esch. coli* B cells without phage particles being detectable. Adsorption of phage particles to *Esch. coli* B cells is followed by reorganization of the cell contents. The fine structure of the protoplasm becomes coarser. Bacteriophage particles appear throughout the cell protoplasm, often in linear aggregates aligned in an interparticle matrix; the appearance of the individual particles varies from those in which structure is clearly defined to those in which definitive structure is suggested only. When *Esch. coli* cells are lysed, certain evidences of pattern may be discernible in the débris. For instance, the cell wall yields many characteristic elliptical and circular segments. In older

preparations cells may be found packed with phage particles. Our observations are in better accord with the conception that the coliphage particles are synthesized by an altered metabolic mechanism of the parent cell than that they multiply by fission or other means.

Discussion

(Dr. Edwin W. Schultz, Stanford University, Calif.) We have been working with another phage, a pyocyanus bacteriophage, and it has been our observation also that the particles of this phage are always of much the same size and we have never been able to observe anything in our micrographs suggesting that they undergo division. However, I wonder how Dr. Mudd would explain the intricate structure, within the head portion, for example, on the basis of an auto-catalytic type of reproduction? It seems to me that this is too complex to be easily explained on such a basis.

(Dr. Stanley H. Durlacher, Edgewood, Md.) Dr. James S. Murphy and I performed a very crude experiment. We thought it might be interesting to take *Esch. coli*, extract them with various fat solvents, acids and alkalis, and study them with the conventional electron microscope. We did this and I believe it was our alkali-treated organisms that showed an inhomogeneity of structure which resembled very much that shown by Dr. Mudd with the improved electron microscope. I wonder whether he has ever done anything of this sort, and whether we are seeing the same thing he did.

(Dr. Mudd) In answer to Dr. Schultz' question. I think that the earlier hypothesis of autocatalytic generation of bacteriophage from precursors already present in the host cell is quite out of accord with many facts which are currently available. On the other hand, I am impressed with two manuscripts by Seymour S. Cohen, at present in press in the Journal of Biological Chemistry, which indicate that infection with coliphage radically diverts the metabolism of the parent cell from the elaboration of *Esch. coli* protoplasm to the synthesis of desoxyribose nucleoprotein characteristic of the infected phage. P_{32} present as phosphate in the external medium was shown to be the principal source of phosphorus for the desoxyribose synthesized. In accord with this our electron microscopic pictures seem to us in much better agreement with the conclusion that phage particles are synthesized in the altered protoplasm of the parent cell than that they result from division of phage particles.

MUCORMYCOSIS, WITH REPORT OF ACUTE MYCOTIC PNEUMONIA. Roger D. Baker and (by invitation) A. O. Severance, Birmingham, Ala., and San Antonio, Texas.

Abstract. A case of acute lobular pneumonia in a child of 3 years in Texas, due to *Mucor*, is reported. Mention is made of another case of mucormycosis of the nares and brain. As 4 cases of mucormycosis have recently been reported in the American literature, 6 cases are available. All have been acutely fatal and have occurred in patients with diabetes mellitus. The fungus produces necrosis and acute inflammation, grows in blood vessel walls, and causes thromboses. A brief summary of the older, chiefly German, literature on mucormycosis is presented.

Discussion

(Dr. Alfred Golden, Memphis, Tenn.) We observed and reported the 3 cases to which Dr. Baker referred, and it seems to me this peculiar infection in the diabetic offers us another tool in the differential diagnosis of non-pathogenic and

pathogenic fungi. We were impressed with the invasiveness of this organism, as Dr. Baker was. We observed in 2 of the cases that the organism could invade tissues as tough as the sclera, and in some cases produced a polymorphonuclear response, and in others it did not. It is commonly assumed that the degree of leukocytic reaction to a fungus is an index of pathogenicity, but I think we have another lesson to learn from Dr. Baker's case, as well as from the others: that we should pay more attention to the invasiveness of a fungus, even if there is little tissue reaction around it.

MELIOIDOSIS. REPORT OF SECOND CASE FROM THE WESTERN HEMISPHERE, WITH BACTERIOLOGIC STUDIES ON BOTH CASES. Parker R. Beamer, and (by invitation) Philip L. Varney, Wilson G. Brown, Frank McDowell, and Birkle Eck, St. Louis, Mo.

Abstract. Melioidosis is a specific, glanders-like infection in human beings, caused by a small, pleomorphic, Gram-negative, rod-shaped bacterium, which resembles the glanders bacillus in some respects while differing from it in other characteristics. In 1912 Whitmore first isolated the causal agent from human cases of melioidosis in Rangoon. Although the organism is classified as *Malleomyces pseudomallei*, it is known also under other names such as *Bacillus pseudomallei*, *B. whitmori*, *Flavobacterium pseudomallei*, *Pfeifferella whitmori*, *Actinobacillus pseudomallei*, and *Loefflerella whitmori*.

Ordinarily, melioidosis is an acute pulmonary infection, with hematogenous dissemination of the organisms to several viscera, producing numerous miliary abscesses, and septicemia followed by death in a few days. Some 300 cases are recorded in the medical literature, approximately two-thirds of these occurring in Rangoon, and the remainder in other areas in the Far East. Only a few cases of chronic melioidosis have been encountered, and usually in individuals who survived the acute form of melioidosis. With one exception all of these patients were believed to have contracted the disease in the endemic region described above.

McDowell and Varney reported a case of chronic melioidosis, believed to be the first one which originated in the Western Hemisphere. This patient had not been out of the United States except for 2 years in Panama, several years prior to the onset of the disease.

The present report concerns a 25-year-old woman member of the U.S. Marine Corps admitted to the hospital with chief complaints of soreness and swelling in the right lower abdomen. Examination revealed tenderness and induration in the right inguinal region. Subsequently this area desquamated, exposing deeper layers of the skin partially covered by serofibrinous exudate. Several tender nodes became apparent in both inguinal regions, and, from time to time, vesicles and bullae developed in the skin. Gradually the lesions in the skin spread to the left inguen. Microscopic examination of a biopsy from this region revealed numerous inflammatory cells in the subcutaneous tissues, chiefly lymphocytes and plasma cells with a few polymorphonuclear leukocytes, but no evidence of a specific causal agent. The skin lesion continued to spread, the patient's general condition grew steadily worse, and she died approximately 9 months after admission, after failure to respond to intensive treatment with sulfadiazine, penicillin, streptomycin, fuadin, and other therapeutic measures.

Post-mortem examination revealed a large chronic abscess, underlying the skin lesions described above and involving the retroperitoneal tissue with necrosis of subcutaneous structures and muscle of the left side up to the perirenal area, posteriorly across the midline to the right side, and downward into the muscles

and subcutaneous tissues of the left thigh. Microscopically, the wall of the abscess was composed of an outer layer of slightly or moderately dense fibrous tissue with several thick-walled blood vessels and numerous inflammatory cells, chiefly lymphocytes, plasma cells, and a few polymorphonuclear leukocytes. The inner portion of the abscess wall was comprised of partially organized fibrinous exudate containing numerous polymorphonuclear leukocytes. Microscopic examination of the liver revealed dissociation of hepatic cords in an advanced degree, necrosis of hepatic cells, and several minute foci of granulomatous inflammation. In the kidney several of the distal convoluted tubules were involved in focal granulomatous inflammation. These visceral lesions may represent the effect of sulfa drug, or they may be associated with the primary infection.

Portions of the wall of the abscess were cultured on blood agar and numerous colonies of poorly staining, Gram-negative, bipolar, motile, pleomorphic rod-shaped bacteria were isolated, in smooth and rough forms. Morphologically, the organisms resembled the glanders bacillus, with the exception of motility, and the colonies resembled those of *M. pseudomallei* described in the literature. After thorough study of morphologic and biochemical characteristics the organism from this case was identified as *M. pseudomallei*. It is almost identical with the strain isolated from the first case. Each organism was agglutinated by the respective patient's serum to a significant titer, and by antiserum prepared with a known strain of *M. pseudomallei*. Complete bacteriologic studies will be reported in a paper now in preparation.

Discussion

(Dr. Joseph F. McManus, Birmingham, Ala.) Were animals inoculated with this material? Mice, I think, are the most important.

(Dr. Beamer) Mice and guinea-pigs were inoculated with the organism isolated from both of these cases. Straus' reaction in a slight degree was observed in guinea-pigs inoculated intraperitoneally. Both strains of the organism were more virulent for white mice. Small amounts of young cultures produced death regularly. Post-mortem examination of infected animals revealed thick, viscid exudate in the peritoneal cavity. Organisms were present in large numbers in this exudate and also in the blood. If animals survived beyond a few days, small focal lesions were noted in the viscera. Cultures from these organs, particularly from the focal lesions, resulted in isolation of the organism.

CYTOPLASMIC INCLUSION BODIES IN INTESTINAL EPITHELIUM OF MICE. RELATION TO DIARRHEAL DISEASE IN SUCKLINGS. Alwin M. Pappenheimer and (by invitation) F. Sargent Cheever, Boston, Mass.

Abstract. In a previous report, Pappenheimer and Enders described the occurrence of intranuclear inclusions in a large proportion of suckling mice with diarrhea. The original stock in which the disease had been prevalent for several years was accidentally destroyed during the summer of 1946. Since then, new stock from several sources has developed diarrheal disease clinically indistinguishable from that occurring in the original stock. In no instance has it been possible to demonstrate intranuclear inclusions of the type described. However, cytoplasmic inclusions, best shown with the Laidlaw acid fuchsin-orange G stain, are present in a high proportion of the suckling mice with diarrhea arising either spontaneously or following the oral administration of extracts of diarrheal intestine. They are limited to the epithelial cells of the small intestine, over the summits of the villi, and are regularly present during the first few days of

the disease. Later, the inclusion-bearing cells are desquamated into the lumen. There is no inflammatory reaction. Normal stock mice, or controls fed with boiled extract, or extract of intestine from normal mice, do not show cytoplasmic inclusions of this type.

Discussion

(Dr. William H. Carnes, Baltimore, Md.) I would like to ask Dr. Pappenheimer whether he has ever looked for similar inclusions in the intestines in cases of infantile diarrhea, and if so, whether he has seen anything that resembles these.

(Dr. Pappenheimer) I regret very much that I have not had opportunity to study human material. One has to make sure of proper fixation, because the superficial epithelium is so easily desquamated after death, and that is where one finds the inclusion bodies in the mouse intestine. Furthermore, if there is any analogy to the mouse diarrhea, one would expect to find inclusions only in the first days of the disease.

READ BY TITLE

THE BLOOD AND BONE MARROW IN PATIENTS WITH CIRRHOSIS OF THE LIVER.

Lawrence Berman and (by invitation) Arnold R. Axelrod, Detroit, Mich.

Abstract. The peripheral blood and bone marrow findings in cirrhosis of the liver have been analyzed on the basis of a review of the literature and the authors' study of 25 patients with diagnoses verified by biopsy of the liver. The principal blood findings are macrocytic or normocytic anemia with normal or elevated mean corpuscular hemoglobin values, lymphopenia, and thrombocytopenia in the majority of the cases. Anemia may be independent of bleeding, and the severity of the anemia or macrocytosis does not appear to be related to the severity or duration of the liver lesion in patients with cirrhosis, although this appears to be true of experimental cirrhosis in rats. The consistent change in the bone marrow is extension of the marrow organ so that active hematopoiesis is found in the shafts of the long bones. Regardless of the presence or absence of bleeding or anemia, the marrow of the sternum is of normal or increased cellularity, with normal or increased erythrocytogenesis and megakaryocytogenesis in most cases. Hypocellularity of the marrow is an unusual finding, even in patients with advanced liver lesions. Macronormoblastic erythropoiesis is seen in patients with macrocytic anemia, but megaloblastic erythropoiesis does not result from hepatic cirrhosis.

The presence of peripheral cytopenias (anemia and thrombocytopenia), in spite of normal or increased formation of erythroblasts and megakaryocytes in the marrow, is suggestive of hypersplenism in patients with cirrhosis of the liver. In patients with chronic hemorrhage the blood and sternal marrow pictures are those of iron-deficiency anemia, although other changes such as lymphopenia and thrombocytopenia tend to persist.

The combined studies of the peripheral blood and sternal marrow are often of value in establishing a diagnosis of cirrhosis of the liver.

SEX DIFFERENCE IN THE ALKALINE PHOSPHATASE DISTRIBUTION IN THE KIDNEY OF THE MOUSE. Thelma B. Dunn, Bethesda, Md.

Abstract. A sex difference was noted in the distribution of alkaline phosphatase in adult male and female kidneys from 5 inbred strains of mice. In both the male and female, the cells of a short segment of the proximal convoluted tubules after leaving the glomeruli were intensely stained throughout. In the male, an

additional segment showed an intense staining of the brush borders only. This difference developed with sexual maturity, and it was not detected in immature mice or in other species examined, namely, the rat, guinea-pig, and rabbit.

THE EFFECT OF SULFATHIAZOLE UPON EXPERIMENTAL PYELONEPHRITIS IN RABBITS. John H. Fisher and (by invitation) N. O. Toplack, London, Ont.

Abstract. Not received.

A HISTOLOGIC AND CHEMICAL STUDY OF NECROSIS OF SKELETAL MUSCLE IN ACUTE ISCHEMIA. John W. Harman and Rodney P. Gwinn (by invitation), Madison, Wis.

Abstract. It was previously observed that after the institution of complete ischemia in the limbs of rabbits and rats a progressive, characteristic necrosis commenced to manifest itself at the fourth hour with the appearance of Bowman's discoid degeneration. A further study revealed that release of the major vascular occlusion failed to preclude extension of the necrosis because of the resultant stasis. In a closely correlated study of the histologic and biochemical changes and the contractility in such muscles, it is found that the extent of the necrosis reaches its maximum by 3 hours subsequent to release of the tourniquet and is proportional to the duration of the ischemia. Contractility rarely reappears until a lapse of 20 hours after release of the tourniquet and the percentage of muscles which are excitable is also dependent upon the duration of the ischemia and the extent of the necrosis.

In muscles ischemic for 4 hours it is known that the high-energy compounds, glycogen, adenylypyrophosphate, and phosphocreatine, are completely hydrolyzed and are not resynthesized within 4 hours of release of the tourniquet. This is substantiated by inability of such muscles to contract upon faradic stimulation. After 24 hours, however, over 80 per cent of such muscles are contractile to faradic stimulation. Chemical analysis reveals that in these there is a significant resynthesis of glycogen, adenylypyrophosphate, and phosphocreatine. Lactic acid also is present in greater quantity than in normal muscle.

It is suggested that the anoxia associated with the ischemia permits depletion of the high-energy reserves, which initiates the collapse of the cellular structure, as indicated by the proportionality of this to the duration of ischemia and by the rapidity with which this is reached after relief of the ischemia. On the other hand, the very considerable structural survival and both biochemical and physiologic recovery of muscle fibers accord most aptly with the view that cell death and "biochemical irreversibility" are not dependent upon depletion of the energy reserves unless this is associated with some more profound alteration. It is believed that this determinant of irreversibility is the structural disintegration which, though the final event in the sequence, is the decisive one. This structural change is seen in several forms to which are applied the designations of Bowman's discoid degeneration, Zenker's hyaline degeneration, and Fishback's granular degeneration, even though all have a similar pathogenesis.

HODGKIN'S GRANULOMA INVOLVING BONE. John B. Hazard, Cleveland, Ohio.

Abstract. Bone involvement in Hodgkin's granuloma at autopsy is rather common, but as a presenting lesion, especially without detectable changes in the peripheral lymph nodes, it is unusual. Recently 2 cases in the latter category were observed. A woman, 47 years of age, with pain and a mass in the sacro-iliac region, on roentgenographic examination, was found to have an osteoblastic lesion of the left ilium and also evidence of a pulmonary mass with enlarged

mediastinal lymph nodes. Biopsy of the ilium revealed typical Hodgkin's granuloma. A man, 45 years old, with the complaint of pain in the chest and back, roentgenographically presented an expanded, rarefied lesion in the 8th rib. The resected segment was replaced almost entirely by semifluid yellowish tan tissue forming an ovoid mass 4 cm. in diameter, with an irregular bony shell. Unlike the usual lesion of Hodgkin's disease, the mass was formed principally by purulent exudate, often in large lakes, and by granulation tissue. The latter was infiltrated by many polymorphonuclear neutrophils, in patches by eosinophils and lymphocytes, and by large mononuclear cells, which were in considerable numbers in some locations and occasionally presented atypism evidenced by variability in size and nuclear vesicularity. The lesion was regarded as a granuloma, probably eosinophilic granuloma. Subsequently, the axillary and inguinal lymph nodes became enlarged and on biopsy were typical of Hodgkin's disease. Though Hodgkin's granuloma has not been authenticated as arising primarily in bone, the osseous lesions may be a primary manifestation of considerable importance in diagnosis of the disease. Softening and suppuration may be prominent pathologic features.

THE TOPOGRAPHY OF CHRONIC GASTRITIS IN OTHERWISE NORMAL STOMACHS.*

Robert Hebbel, Minneapolis, Minn.

Abstract. Ninety-seven stomachs, free of ulcer, scar, tumor, and obscuring post-mortem changes, obtained at autopsy from individuals of all ages and both sexes, whose past histories recorded no gastric complaints, were searched in sections of rolls of mucosa from the entire lesser and greater curvatures, respectively, for evidences of chronic gastritis. The presence, severity and distribution of lymphocytic infiltration, lymph follicles, atrophy, intestinal metaplasia, pseudopyloric glands, cysts and erosions were recorded. Any parenchymal lesion was considered abnormal. Lymphocytic infiltration in excess of mild degree was considered abnormal, but those specimens which showed only excess infiltrate were kept separate. The changes were noted to be focal (isolated parenchymal lesions well within a microscopic field), patchy (several somewhat larger lesions), or diffuse (involving the whole segment considered).

Abnormality in some degree was found in 70 (72 per cent) of the specimens. Of the 27 stomachs free of changes, 20 were from persons less than age 51 and 7 were from those over age 50. There was no uniformity of involvement between antrum and body, and in either segment the changes varied from isolated foci to diffuse alterations. Some specimens showed focal or patchy lesions on one or both curvatures. A few showed diffuse changes on one curvature and focal or patchy lesions on the other. Some showed diffuse changes on both curvatures, but these made up a small proportion of the total. Many of the lesions were not quantitatively significant but where, short of diffuse involvement, to draw a line between normal and abnormal on the basis of quantitative change is uncertain.

The antrum and body are best considered separately. The antrum was abnormal in some degree in 64 specimens (66 per cent of the total, 50 per cent of the 44 specimens from persons less than age 51, 89 per cent of the 53 specimens from those past age 50). Focal lesions predominated in the younger group. Diffuse parenchymal changes were found in 8 specimens (8.2 per cent of the total), *i.e.*, in 1 (2.3 per cent) of those from persons under age 51 and in 7 (13 per cent) of those from persons over age 50.

The body mucosa was abnormal in some degree in 48 specimens (49.5 per

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

cent of the total, *i.e.*, 27 per cent of those less than age 51, 68 per cent of those over age 50). Diffuse parenchymal changes were found in 14 specimens (14.4 per cent of the total) and thus occurred in 3 (6.8 per cent) of those less than age 51 and in 11 (20 per cent) of those over age 50. Diffuse parenchymal changes involving both antrum and body were observed in but one specimen.

THE EFFECT OF LOW PROTEIN AND LOW CHOLINE DIETS ON THE ABSORPTION OF IRON AND COPPER. D. M. Hegsted (by invitation), T. D. Kinney and J. A. Cartaya (by invitation), Boston, Mass., and Cleveland, Ohio.

Abstract. Rats were fed on a diet low in protein (8 per cent casein) and choline but adequate in vitamins and minerals, to which was added 2.2 per cent ferric citrate or 0.1 per cent copper sulfate, or the two salts together. Iron was definitely toxic as judged by the gross appearance, survival time, and weight loss. Copper was not toxic, but the combination of iron and copper was more toxic than iron alone. Choline did not protect against this toxicity, but the further addition of protein prevented all evidence of toxicity. The nature of the toxic action of iron is not clear. The iron content of the livers was slightly elevated and some iron could be demonstrated in the liver cells on histologic examination, but it is believed that this was not great enough to be the cause. Lack of phosphate absorption was apparently not involved since there was no significant difference in the amount of bone ash between animals receiving iron and control animals. Examination of the bone also showed no variation from the control animals.

Choline and copper appeared to be interrelated. Copper largely prevented fat deposition in livers of rats on choline-deficient diets. Further, when choline was added to the diet larger quantities of copper were present in the liver.

THE PATHOLOGIC EFFECTS OF TWO PHOSPHINE OXIDE ANTICHOLINESTERASES. H. Walter Jones, Jr., and Benjamin Landing (by invitation), Bethesda, Md.

Abstract. During investigation of the relationship between the toxicity of organic phosphorus compounds and their inhibition of brain cholinesterase *in vitro*, it was discovered that p-chlorophenyl diethoxy phosphine oxide (A) and bis (p-chlorophenyl) ethoxy phosphine oxide (B) significantly inhibit cholinesterase, A being over ten times as active as B. However, deaths resulting from a single injection of either compound frequently were delayed as much as 5 days, whereas the anticholinesterases di-isopropyl-fluorophosphate and tetraethyl pyrophosphate cause immediate deaths only. Pathologic effects following intraperitoneal administration of these compounds were therefore studied in mice, rats, dogs, and rabbits.

The lesions produced by both compounds in the various organs were essentially the same in all animals. The splenic follicles showed pyknosis and degeneration of lymphocytes, and phagocytosis of debris by large reticulum cells; larger doses produced hypocellularity without phagocytosis in both white and red pulps. After 2 days the mitosis rate in the follicles tended to increase. The thymus showed widespread pyknosis and degeneration of the cortical lymphocytes. The changes produced in lymph nodes and Peyer's patches consisted of lymphocytic degeneration and phagocytosis of debris after smaller doses, and more marked lymphocytic degeneration and hypocellularity without phagocytosis after larger doses. Congestion and mild hypocellularity were the only changes observed in the bone marrow.

In mice, the superficial protoplasm of the renal proximal convoluted tubules sloughed into the lumina, forming protein casts. In rats, this cytoplasmic slough

did not occur, but the nuclei of the epithelial cells of the proximal convoluted tubules were often pyknotic. In the gastrointestinal tract, increased mucous secretion and an increased number of mitotic figures in the epithelium of the glands were observed. In the testis, pyknosis of all of the spermatogenic layers with shrinking or loosening of the layers was the only change observed. In a few animals with relatively severe lymphoid damage, the Kupffer cells of the liver were pyknotic. The lungs during the first 48 hours showed moderate atelectasis, with increased leukocytes in the alveolar capillaries, and congestion. The brain after B showed mild edema and shrinkage of some cortical ganglion cells. Peripheral (sciatic) nerve was examined in one dog after three injections of B over a period of 20 days. No evidence of peripheral neuritis was observed. Compound A produced microscopic evidences of peritonitis, perisplenitis, and peripancreatitis; these did not occur with B.

The total and differential leukocyte count were unaffected by single LD_{50} doses of A or by six injections of one-half LD_{50} each during an 8-day period, but doses of 2.5 LD_{50} caused an absolute lymphopenia and usually a polymorphonuclear leukocytosis.

The sulfur present in the urine as ethereal sulfate was elevated after injections of A in rats and rabbits, suggesting that these compounds are excreted as sulfate conjugates.

Visceral lesions of the type described are not observed following lethal doses of more active anticholinesterases, so that these compounds may have some other mode of action. The pattern of organs damaged, lymphoid tissues, testis, kidney, and gastrointestinal tract, suggests that seen with mustards, but the effects are much less severe than those produced by many mustards at comparable dose levels and seem inadequate to account for death. The doses of these two compounds necessary to damage proliferating tissues are so toxic that they appear to have no potential use in tumor therapy.

DEVELOPMENT AND PATHOGNOMONIC EVALUATION OF GIANT CELLS IN BONE TUMORS AND SIMILAR CONDITIONS. Fritz Levy, Huntington, W. Va.

Abstract. The term "giant cell" is used for two different formations which are remarkably larger than the average or standard cell of a certain tissue. The first one is characterized by unsharp or sharp limitation of the cytoplasm and a number of nuclei of the same size. This formation is represented in normal tissue by the so-called osteoclast. The second type includes polyploid cells with 1 or more nuclei with increased chromosome number; it is represented in normal tissue by the megakaryocyte of the bone marrow.

Both kinds of formations are found in various neoplastic diseases. They do not have special functions, but, if they have any functions at all, these are remainders of the functions of the cells of origin. The different ways of development show that only the presence of numerous polyploid cells is an essential factor in the diagnosis of malignancy, since single polyploid cells occur occasionally in all tissue.

BRUCELLOTIC OSTEOMYELITIS OF THE SPINAL COLUMN IN MAN. Leo Lowbeer (by invitation), Tulsa, Okla.

Abstract. It is well recognized that *Brucella* organisms not only cause septicemia but also focal inflammations, often of granulomatous character, in man and other animals. Osteomyelitis of the spine and other bones of the hog caused by *Brucella suis* has been described by Feldman, Graham, and others, and I have

studied its histologic character. Osteomyelitis can be produced in about 30 per cent of *Brucella*-inoculated guinea-pigs. In man approximately 200 cases of brucellic osteomyelitis have been described by roentgenologists and clinicians, three-fourths of which showed involvement of the spine. These were caused predominantly by the melitensis strain. The low mortality of brucellosis partly accounts for the almost complete absence of microscopic studies on brucellic osteomyelitis.

Through the courtesy of Dr. T. de Villafane Lastra, Professor of Epidemiology at the University of Cordoba, Argentina, I have been fortunate enough to obtain three human spines from patients who died with subacute brucellosis, melitensis type, and who had developed clinical symptoms of spondylitis. The gross specimens showed small and large areas of destruction of disks and contiguous vertebral bodies in the dorsolumbar spine. Occasionally, small osteomyelitic foci were found in anterior or central portions of vertebrae. Exostoses were frequent. Microscopic examination showed subacute osteomyelitis with destruction of cancellous bone, end-plate, cartilage-plate and disk by granulation tissue which in early phases consisted of polymorphonuclear leukocytes, lymphocytes, and plasma cells, and enclosed small abscesses, the contents of which have a tendency to become necrotic. In this phase the process is similar to that found in pyogenic osteomyelitis of the spine, but less destructive, less purulent, and perhaps with more tendency to repair. It also resembles experimental osteomyelitis produced in *Brucella*-inoculated animals.

In later stages large mononuclear cells, fibroblasts, and occasional bizarre multinucleated giant cells of foreign body type appeared. Still later the granulation tissue consisted of large macrophagic histiocytes, surrounded by thick layers of lymphocytes and plasma cells which in turn were surrounded by young fibroblasts and connective tissue. It was well vascularized but underwent extensive central coagulative necrosis of perhaps allergic origin. It contained large numbers of multinucleated giant cells of foreign body type, apparently osteoclastic in nature, surrounding or enclosing small bony sequestra. There was also formation of tubercle-like granulomatous nodules composed of histiocytes of non-epithelioid appearance. Acid-fast bacilli could not be demonstrated. In this phase the process resembles *Br. suis* osteomyelitis of man as described by me, and also spontaneous brucellic osteomyelitis of the hog. Necrosis, therefore, occurs in melitensis as well as in suis infections.

Whether or not these lesions are actually caused by *Brucella* cannot be stated with absolute certainty as long as cultures taken directly from the affected vertebrae are not available. Since, however, these lesions occurred in cases of active subacute brucellosis with positive blood cultures; since, in the absence of fistulae, there was no likelihood of secondary bacterial invasion; since the absence of a truly suppurative inflammation in the early phase speaks against infection by pyogenic organisms; and since the later granulomatous-necrotizing phase bears no true resemblance to tuberculous infection, one may safely assume that the lesions are brucellic in character.

PATHOLOGY OF RUPTURE OF THE SPLEEN IN ACUTE VIVAX MALARIA. Joseph M. Lubitz, Wood, Wis.

Abstract. A pathologic study was made of the ruptured spleen in acute vivax malaria. Four cases were available for examination, in 3 of which *Plasmodium vivax* was isolated and in the fourth the diagnosis of malaria was highly probable. Rupture of the spleen in malaria is preceded by a subcapsular hematoma. Char-

acteristically, the microscopic picture is that of diffuse reticulum cell hyperplasia, subendothelial and adventitial leukopoiesis, and dilatation of sinusoids and venules. Thrombosis and infarction may occur, but were not found consistently. In all cases there was subcapsular dilatation of vessels with small hematmata, not only at the site of rupture, but also in distant areas. Extension of such hemorrhages to the capsule and their confluence appeared to produce the subcapsular hematoma. Stagnation of blood in the sinuses is believed to be caused by the general cellularity and the narrowing of the lumina by subintimal leukopoiesis. As stated by Rigdon, this is a contributing factor in the formation of thrombosis and infarction. Clinically, a pleural effusion on the left side may indicate splenic rupture.

THE STRUCTURE OF THE RENAL GLOMERULUS IN THE NORMAL HUMAN KIDNEY AND IN SOME DISEASE CONDITIONS.* J. F. A. McManus, Birmingham, Ala.

Abstract. 1. The basement membrane in the normal glomerulus as shown with the periodic acid-Schiff's reagent technic derives from Bowman's capsule and attaches to the arterioles at the glomerular root. It encloses capillary loops and certain infrequent stroma cells of the mesangium, and the latter in the intercapillary or axial space. 2. The axial space is prominent in diabetes mellitus. It appears finely fibrillar. 3. Marked involvement of the axial space can be seen in various disease conditions: acute inflammation in acute glomerulonephritis; glycoprotein accumulation in intercapillary glomerulosclerosis; vacuolation and reticulation in eclampsia; lipid accumulation in "lipoid nephrosis."

FACTORS INFLUENCING COLLAGEN CONTENT IN EXPERIMENTAL CIRRHOSIS.* Thomas G. Morrione (by invitation), Burlington, Vt.

Abstract. Cirrhosis was produced in 220 rats by exposure to carbon tetrachloride vapors every other day for 35 days. The increase in hepatic collagen, as determined quantitatively by Lowry's method, followed a curve of exponential type. After stopping the carbon tetrachloride, significant decreases occurred in hepatic collagen. The decrease was greatest on a low protein diet supplemented with methionine, choline, and cystine. Little or no collagen resorption occurred on a low protein-high fat diet. Ligation of the portal vein in rats with cirrhosis interfered with resorption of collagen provided no adhesions were present. The latter favored reversal of the cirrhosis.

HISTOLOGIC LESIONS ENCOUNTERED IN SEGMENTAL ENTERITIS. Henry Rappaport and Fred H. Burgoyne (by invitation), and Hans F. Smetana, Washington, D.C.

Abstract. A detailed histopathologic study of 110 cases of segmental enteritis submitted to the Army Institute of Pathology between the years 1940 and 1947 revealed certain histologic features which could not be explained readily on the basis of ulceration and chronic inflammation alone. These were: (1) edema out of proportion to the severity of the inflammatory infiltration; (2) marked dilatation of the lymph vessels; (3) presence of granulomatous lesions having the morphologic features of non-caseating tubercles in the intestinal wall and in the regional lymph nodes.

Granulomas with giant cells were found in the intestines in 40 (36 per cent) of the cases and were of two main types. One was characterized by predominance

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

of giant cells of the foreign body type, did not appear well organized, and usually blended imperceptibly with the surrounding inflammatory reaction. The giant cells frequently contained foreign bodies of varied structure, the nature of which usually could not be identified. The second type of granuloma consisted of well organized, circumscribed masses of epithelioid cells, with or without giant cells, and arranged in a tubercle-like fashion, histologically indistinguishable from the non-caseating, tubercle-like lesion observed in sarcoidosis. This type occurred in the intestines in 21 (19.1 per cent) of the cases. Lymph nodes were available for study in 17 of these 21 cases and 16 of them showed identical non-caseating, tubercle-like granulomas. In none of these cases, however, was there clinical or autopsy evidence of generalized sarcoidosis. In comparison, none of the 22 autopsied cases of sarcoidosis on file at the Army Institute of Pathology showed any involvement of the jejunum or ileum.

An analysis of the racial distribution of segmental enteritis and sarcoidosis revealed the following:

<i>Disease</i>	<i>White per cent</i>	<i>Negro per cent</i>
Segmental enteritis (all cases).....	94.4	5.6
Segmental enteritis with non-caseating tubercle-like granulomas	88.9	11.1
Sarcoidosis	39.4	60.6

Vascular changes were encountered in a considerable number of cases: Among these, marked endarteritis was seen most frequently. The intimal proliferation was sometimes confined to a limited segment of the vessel. In a few instances both granulomatous arteritis and phlebitis were observed. In some cases there was a peculiar type of granulomatous lymphangitis in which the granulomas encroached upon and obstructed the lumina of lymph vessels.

EXPERIMENTAL ENDOMETRIOSIS. Jacob M. Ravid, New York, N.Y.

Abstract. This problem was undertaken for the purpose of learning something about the pathogenesis of endometriosis. Forty rabbits were used. Pieces of endometrium were excised and then implanted in the skin, peritoneal cavity, ovary, and the anterior chamber of the eye. Some of the animals subsequently received injections of estrogens and of urine from pregnant women. After varying periods of time positive "takes" were obtained in about 60 per cent of the animals. Such lesions were nodular and cystic. Microscopically, these nodules were made up of islands of endometrium, some of which had the typical appearance of miniature uterine cavities. Some of them also showed proliferation of smooth muscle fibers and embryonal cytogenic stroma. These experiments demonstrate the comparative ease with which experimentally implanted bits of endometrium can be made to grow in the rabbit, thus lending support to Sampson's implantation theory of endometriosis.

OBSCURE AXILLARY LYMPH NODE METASTASIS IN CARCINOMA OF THE BREAST. Otto Saphir and (by invitation) George D. Amromin, Chicago, Ill.

Abstract. Axillary lymph nodes from 30 patients with carcinoma of the breast, which on routine examination were reported as uninvolved, were restudied histologically by means of serial sections. Of these 10, or 33.3 per cent, contained

carcinoma cells. No relationship could be established between a hyperplasia of the sinus endothelium or of the reticulum cells, or of so-called pre-invasive changes and the presence or absence of metastases in lymph nodes. The necessity of performing more careful and thorough examinations of nodes in regions of malignant tumors is emphasized. The only means of ruling out carcinoma metastases is examination by serial sections. These are essential for correct prognosis and evaluation of surgical results.

MORPHOLOGIC CHANGES IN SYPHILITIC LESIONS DURING THE HERXHEIMER REACTION. Walter H. Sheldon and (by invitation) Albert Heyman, Atlanta, Ga.

Abstract. The Jarisch Herxheimer reaction has long been recognized as a complication of the treatment of syphilis. This reaction is a unique phenomenon since it occurs only in response to antisyphilitic therapy. It is thought to be caused by the release of spirochetal breakdown products. The syphilitic lesions frequently show gross changes during this reaction, but no histologic observations have been reported.

We have made histologic studies of the cutaneous and mucosal lesions during the Herxheimer reaction in several groups of patients with secondary syphilis. Definite histologic changes occur during this reaction. These consist of congestion, edema, alteration of the vascular endothelium, and acute inflammatory cellular infiltration. The changes are confined strictly to the syphilitic lesions. They appear within 5 hours after treatment and subside within 18 to 24 hours. These histologic changes were found in practically all patients with clinical evidences of the Herxheimer reaction. Similar changes probably occur during the Herxheimer reaction in late syphilitic lesions of the cardiovascular and central nervous system.

Our findings show that histologic changes occur in syphilitic lesions during the Herxheimer reaction. These changes may account for the serious complications which are occasionally encountered. Our findings also reveal a similarity between the morphologic changes of the Herxheimer reaction and hypersensitivity of the tuberculin type. Further studies are in progress which may lead to a better understanding of some of the immunologic aspects of syphilitic infection.

THE PULMONARY MANIFESTATIONS OF SCLERODERMA: AN ANATOMIC-PHYSIOLOGIC CORRELATION. David M. Spain and (by invitation) Albert G. Thomas, New York, N.Y.

Abstract. Among the visceral changes occurring in scleroderma, those in the lung may lead to serious clinical results. The changes consist of hyaline fibrotic thickening in the interstitium of the lung parenchyma. Numerous cystic areas are present as well as compact areas of fibrosis. Many alveoli are gradually reduced in size. Associated bronchiolar changes give rise to patchy zones of obstructive emphysema. A case is presented with detailed respiratory and ventilatory functional studies. These studies are correlated with the necropsy findings. Both the respiratory and ventilatory functions are markedly impaired. The anatomic changes responsible are: Involvement of skin over the thorax with impairment of chest motion; fibrous contraction of the pleura with resultant compression of the lung; diffuse peribronchiolar fibrosis with obstructive emphysema; and diffuse interstitial fibrosis with impairment of gaseous exchange. At times these lung changes may be the first or the most prominent manifestation of scleroderma.

HETEROTOPIC BONE FORMATION IN THE SKIN. Robert E. Stowell, St. Louis, Mo.

Abstract. Nine instances of heterotopic bone formation in the skin are reported. Two occurred in basal cell carcinomas. Step sections through 40 healing or healed surgical scars revealed bone in 3 specimens. The findings in these cases, together with those in 100 similar cases collected from the literature, are discussed in considering probable etiologic factors in the formation of heterotopic bone.

DIFFERENTIAL DIAGNOSIS IN CONGENITAL SYPHILIS OF THE UMBILICAL CORD.*

Ruth C. Wanstrom and A. James French, Ann Arbor, Mich.

Abstract. Not received.

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THE EFFECT OF PATENT DUCTUS ARTERIOSUS AND OF INTER-AURICULAR AND INTERVENTRICULAR SEPTAL DEFECTS ON THE DEVELOPMENT OF PULMONARY VASCULAR LESIONS *

KENNETH J. WELCH, M.D.,† and THOMAS D. KINNEY, M.D.‡

(From the Departments of Pathology of the Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Mass.)

During the past several years there has been increasing interest in the diagnosis and surgical treatment of congenital heart disease. No systematic study has been made of the possible changes in the pulmonary circulation in cardiac anomalies in which there is a left to right shunt. This matter now assumes greater importance as the result of operations recently introduced in which a systemic vessel is anastomosed to the pulmonary artery. For this reason, representative groups of cases of congenital heart disease in which there was a left to right shunt were studied. The histologic changes in the lungs were evaluated and an attempt was made to correlate these findings with known physiologic facts regarding the pulmonary circulation in similar groups of cases.

Material was gathered from the autopsy files of the Beth Israel (BIH), the Children's (CH), the Mallory Institute of Pathology at the Boston City (BCH), the Massachusetts General (MGH), and the Peter Bent Brigham (PBBH) Hospitals. This represented an autopsy population of 44,220. From this group, 67 cases of congenital heart disease, considered to be suitable for this study, were selected.

Only cases which were significant from a clinical as well as a pathologic viewpoint were used. Cases in which there were multiple defects were discarded unless the associated lesions might be expected to increase the degree of left to right shunt. Three main groups were studied: (1), patent ductus arteriosus; (2), interauricular septal de-

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† Now at Children's Hospital, Boston, Mass.

‡ Now at Cleveland City Hospital, Cleveland, Ohio.

fects; and (3), interventricular septal defects. A fourth, but smaller group in which there was a combination of lesions giving a left to right shunt also were included in the study.

Control groups of 10 cases from each of the first 7 decades of life were studied also to give a baseline of pulmonary vascular change resulting from age alone. Care was taken to avoid, so far as possible, any condition which would predispose to secondary pulmonary vascular change. Special care was taken to eliminate cases in which there was chronic pulmonary disease, *i.e.*, emphysema, extensive fibrosis, obliterating pleuritis, tuberculosis, neoplasm, or thoracic deformity. Cases with cardiovascular-renal disease of any type also were discarded.

METHODS

It was necessary to depend upon autopsy protocols for the description of lesions of the large branches of the pulmonary artery. This represented the work of a large number of prosectors with consequent variation in reliability. Routine sections of lung were used for microscopic study. These usually were taken at random from unspecified portions. Two to eight blocks from each case were studied. Sections were fixed in either Zenker's acetic or 10 per cent formalin solutions. They were stained with hematoxylin and eosin, a combination of van Gieson's and Weigert's elastic tissue method on the same section, and Masson's trichrome light green stain.

Because of the confusion in terminology regarding the divisions of the pulmonary artery, the vessels were arbitrarily divided by size into four groups. External diameters of the arteries were computed from the external elastic lamellae. Group I consisted of vessels greater than 1 mm. in diameter; group II, 250 to 500 μ ; group III, 100 to 250 μ ; and group IV, 25 to 100 μ . These were studied to determine the type, location, and extent of any vascular lesions present.

The sections were pooled and then examined objectively without knowledge of the age of the patient or the extent and nature of the cardiac lesions.

The changes were graded from 1 plus to 4 plus depending upon the severity of the lesion. The lesions in each group were classified under three general headings: intimal proliferative changes, hyaline changes, and medial changes (Table I).

The intimal proliferative lesion consisted of an increase in subendothelial connective tissue and was frequently associated with splitting and reduplication of the elastica interna. This change ranged from

asymmetric plaques involving a small segment of the circumference of a vessel in mild lesions to a total obliterating endarteritis in severe lesions. There was considerable variation in the cellularity of the lesions, and occasionally vacuolar degeneration was found. There appeared to be a transition stage between intimal proliferative and intimal hyaline lesions with loss of cellularity and increasing evidence of collagen deposition.

Hyaline lesions consisted of a deposition of acellular, homogeneous, subendothelial hyalin. In the mild lesions, small asymmetric deposits were present, while in severe lesions a thick hyaline ring was found with marked reduction of the lumen of the vessel. The location of the

TABLE I
Grading of Lesions

	+	++	+++	++++
Intimal proliferation	Occasionally present and asymmetric	Consistently present but asymmetric	Consistently present and uniform	Marked reduction to obliteration of lumen
Hyalin	Occasionally present and asymmetric	Consistently present but asymmetric	Consistently present and uniform	Marked reduction to obliteration of lumen
Medial thickening	Occasional asymmetric thickening	Consistent asymmetric thickening	Uniform thickening	Marked hypertrophy or hyalinization

hyaline material in relation to the internal elastic lamella varied considerably. Usually the hyalin was between the internal elastic layer and the lining endothelium. In some instances it enveloped the elastic lamella, while less commonly it extended for variable distances into the media. This material took an acidophilic stain with hematoxylin and eosin and with the combined Weigert elastic tissue and van Gieson method. With Masson's trichrome stain the hyalin appeared pale green.

The medial layers were examined for evidence of hypertrophy or an increase in number of smooth muscle cells, and for an increase in intercellular collagen. The lesions graded as 1 plus were those in which thickening was asymmetric and present in an occasional vessel, while the lesions regarded as 4 plus were those in which there was marked and consistent concentric hypertrophy or hyalinization of the arteries.

The capillaries and veins were examined to determine the presence or absence of thickening or scarring.

TABLE II
Control Group

Decade	Case	Pulmonary vascular lesions Microscopic											
		1 mm.			250-500 μ			100-250 μ			25-100 μ		
		I*	H†	M‡	I	H	M	I	H	M	I	H	M
1st	1
	2
	3
	4
	5
	6
	7
	8
	9
	10	+	.
2nd	11
	12
	13
	14	++	++	.	++	+	.
	15
	16
	17
	18	+	.
	19	+
	20	+	.	.	+	.	.
3rd	21	—	—	—	.	.	.	+	.	.	+	+	.
	22	+	.	.	+	.	.	+	++	.	.	+++	.
	23	+	.	.	+	.	.	+	+	.	.	+++	.
	24
	25	.	.	.	+	.	.	+	.	.	.	+	.
	26	+	.
	27	+	.	.	.	++	.
	28	+	.
	29	+	.	.	++	.
	30	+	.
4th	31	.	.	.	+	.	.	++	++	.	+	+	.
	32	+	.	.	++	.
	33	.	.	.	+	+	.	+	+	.	.	+	.
	34	++	.	.	++	+	.	++	+	.	.	+	.
	35	+	.	.	+	.
	36	+	.	.	+	.
	37	+	.	.	+	.
	38	++	.	.	+	.
	39	.	.	.	++	.	.	++	++	.	+	++	.
	40	.	.	.	+	.	.	++	+	.	+	++	.
5th	41	+	.	.	+	.	.	++	+	.	.	++	.
	42	.	.	.	+	.	.	+	.	.	.	+	.
	43	+	.	.	++	.	.	+++	+	.	.	++	.
	44	.	.	.	+	+	.	+	++	.	.	+++	.
	45	+	+	.	.	++	.
	46	.	.	.	+	.	.	+	+	.	.	+	.
	47	+	.	.	+	+	.
	48	++	.	.	+	.
	49	+	+
	50	+	.	.	+	.	.	.	++	.	.	++	.

TABLE II (cont'd.)

Decade	Case	Pulmonary vascular lesions Microscopic											
		1 mm.			250-500 μ			100-250 μ			25-100 μ		
		I*	H†	M‡	I	H	M	I	H	M	I	H	M
6th	51	++	.	.	+	+	.	+	+++	.	.	+	.
	52	.	.	.	+	+	.	+	+
	53	.	.	.	+	.	.	+	+	.	+	++	.
	54	—	—	—	+	.	.	++	.
	55	++	.	.	++	+	.	+++	++	.	++	+++	.
	56	—	—	—	++	.	.	++	++	.	+	++	.
	57	.	.	.	++	+	.	+	++	.	+	++	.
	58	+	.	.	+	+	.	++	++	.	.	+	.
	59	+	.	.	+	.	.	++	++	.	.	+++	.
	60	+	.	.	++	.	.	+	++	.	+	+	.
7th	61	—	—	—	+++	+	.	+++	++	.	+	+++	.
	62	.	.	.	+	.	.	+	+	.	.	+	.
	63	.	.	.	+	.	.	++	+	.	.	+	.
	64	.	.	.	+	.	.	++	++	.	.	+	.
	65	+	.	.	+	+	.	.	+++	.	.	+	.
	66	+	.	.	+	+	.	+++	++	.	++	+++	.
	67	+	.	.	++	+	.	+++	++	.	++	+++	.
	68	.	.	.	+	+	.	++	+++	.	.	++	.
	69	.	.	.	++	+	.	.	++	.	.	++	.
	70	+	.	.	+	.	.	++	+	.	++	+++	.

* Intimal proliferation.

† Hyalin.

‡ Medial thickening.

Vascular Changes in Normal Lungs

Vascular changes in the lungs of the control groups were graded according to the criteria used for the groups in which there was congenital heart disease.

A study of Table II, in which the pulmonary vascular lesions in the control group are summarized, indicates that in the first decade of life only one case had a recognizable lesion. In the second decade only scattered minimal lesions were found. In the third decade one case had no lesions, 7 had minimal lesions, and 3 plus lesions were found in the precapillary vessels in 2 cases. It was not until the fourth decade of life that consistent vascular changes were encountered, and these changes, for the most part, were mild. Lesions from this period on were more pronounced, and by the seventh decade 2 plus and 3 plus lesions were encountered consistently in arteries measuring from 25 to 500 μ in diameter.

No changes were found in the capillaries or veins in any of the cases in the control group. No thickening of the capillary basement membrane or change in the alveolar lining cells was observed. Such changes were described by Parker and Weiss¹ in patients having severe mitral stenosis.

The incidence of arteriosclerosis of the pulmonary arteries is remarkably high. In this series some degree of change was found in every case in which the subject was over the age of 40 years and in 9 of 10 cases from the third decade of life. It should be noted again that these cases were carefully selected so as to conform as closely as possible to the normal. It is obvious that any lesions found in the lungs in cases of congenital heart disease must be judged in the light of changes associated merely with ageing.

These findings are in accord with a survey by Brenner² who reviewed 100 consecutive autopsies and found macroscopic evidence of pulmonary vascular sclerosis in 70 per cent. On microscopic study this incidence increased to 97 per cent. In but 3 cases, all under 10 years of age, were no sclerotic changes found in the pulmonary vascular bed.

Patent Ductus Arteriosus

Twenty-five cases of patent ductus arteriosus, considered suitable for this study, were selected from the autopsy material available. Only those cases were used in which the internal diameter of the ductus was greater than 3 mm. The size of the defects ranged from 3 to 15 mm. and 7 measured 10 mm. or more in diameter. Twenty-three of these cases were not complicated by any significant cardiac or vascular anomalies. One case was associated with moderate coarctation of the aorta, and there was marked hypoplasia of the aorta distal to the ductus in the other case. The ages of the patients varied from 2 months to 65 years, with 13 over 20 years of age. There were 9 females and 16 males. The classical clinical picture with a typical "machinery" murmur was present in 17 patients. These cases are summarized in Table III.

Examination of Table III shows that with but one exception the changes in the pulmonary vascular system were no greater than the changes found in the control group in comparable ages. It should be emphasized that no medial changes were found in any of the vessels examined. There were severe intimal proliferative and hyaline changes in one case (BCH no. A-43-64), that of a 37-year-old male who had a ductus with an internal diameter of 12 mm. Four plus intimal proliferative and 3 plus hyaline changes were encountered in vessels measuring from 25 to 100 μ , while in vessels of other sizes the changes were no greater than those found in the control group. This case has been reported in detail by Chapman and Robbins.³ A similar case has been reviewed by Keys and Shapiro.⁴ Their patient was a 48-year-old woman who had a ductus measuring 15 mm. in diameter. The heart weighed 700 gm. and there was marked right ventricular hypertrophy.

The large branches of the pulmonary artery were dilated and microscopically there was marked intimal atherosclerosis of the large and small branches of the pulmonary artery. Other reports of pulmonary atherosclerosis in patients with a patent ductus either have described changes limited to the immediate vicinity of the ductus, have included significant associated cardiac defects, or were difficult to evaluate because of inadequate pathologic study. Isolated reports have noted macroscopic changes but failed to give detailed microscopic descriptions of the nature, distribution, and severity of the pulmonary vascular lesions.

The rarity of reports of cases of patent ductus with pulmonary atherosclerosis and the fact that only one of 25 cases in this series had excessive pulmonary atherosclerosis indicate that such findings are unusual. It should be noted, however, that in both of the reported cases there appeared to be a significant pulmonary vascular block with subsequent right heart failure.

In contrast to the above was case PBBH no. A-44-102, a woman of 27 years with a ductus having an internal diameter of 15 mm. together with a severe diffuse hypoplasia of the aorta beyond the ductus. Evidence that this hypoplasia produced a considerable resistance in the systemic circuit is offered by the fact that immediately after the ductus was divided the left heart dilated and the patient expired on the table. This combination of lesions would necessarily result in a tremendous volume flow through the pulmonary circuit. The lungs showed no vascular lesions.

It has been postulated frequently that there is a significant elevation of pulmonary arterial pressure in cases of patent ductus. Dexter and his group⁵ have measured the pulmonary arterial pressure by means of the venous catheter in 12 patients having patent ductus arteriosus. In 9 cases it was not significantly elevated in the absence of congestive failure. In 3 patients the pressure was elevated despite the absence of clinical manifestations of cardiac failure. However, each had decreased exercise tolerance. The volume flow through the pulmonary artery in these patients was 16.9, 14.2, and 8.8 liters per minute, respectively. Cournand⁶ has recently reported the case of a 3-year-old girl with a patent ductus, who had a pulmonary flow of 5 liters per minute and a systemic flow of 2 liters per minute. Her pulmonary arterial pressure was 55/39 mm. of Hg, which Cournand considered three times normal for the age. In 9 of the 12 cases studied by Dexter the internal diameter of the ductus was greater than 7 mm. One patient, a man of 38 years, had a ductus with a diameter of 13 mm. and a volume flow of 9 liters per minute, yet the pulmonary arterial pres-

TABLE III
Patent Ductus Arteriosus

Autopsy	Age	Sex	Internal diameter of ductus	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
	<i>years</i>		<i>mm.</i>	<i>gm.</i>	<i>mm.</i>	<i>mm.</i>		
CH A-35-4	2/12	M	3	22	4	6	None	Normal
CH A-36-11	3/12	F	3	20	2	6	None	Normal
CH A-39-31	4/12	F	15	Not given	6	10	None	Agenesis of right pulmonary vessels
CH A-31-74	9/12	M	Patent	29	3	8	None	Normal
CH A-39-117	10/12	M	5	47	3	9	None	Normal
CH A-46-131	² 11/12	M	5	88	3	10	None	Normal
CH A-35-104	⁷ 2/12	M	3	180	4	13	None	Normal
CH A-30-132	⁷ 7/12	M	3	129	2	9	None	Normal
CH A-43-187	¹⁰ 10/12	M	10	Normal	4	16	Mycotic aneurysm of pulmonary conus proximal to ductus	Plaques only in vicinity of ductus
PBBH A-43-98	13	M	12	"Slight cardiac enlargement"	10	24	None	Normal
PBBH A-42-142	14	F	4	400	7	20	S.b.e. of ductus, aortic and mitral valves	Normal
PBBH A-39-187	15	F	10	Not given	8	18	S.b.e. of ductus	Normal
PBBH A-44-127	20	M	5	730	4	19	S.b.e. of mitral and aortic valves	Plaques opposite opening of ductus
PBBH A-45-74	21	F	Patent	520	6	19	S.b.e. of ductus	Pulmonary artery markedly dilated
MGH 9237	21	M	5	550	5	15	S.b.e. of pulmonary artery, pulmonary and mitral valves, and ductus	Normal
MGH 8789	21	F	3	380	3-5	14	Slight coarctation and s.b.e. of aortic and mitral valves	Normal

Pulmonary vascular lesions												Blood pressure	Signs and symptoms referable to ductus	Cause of death
Microscopic														
1 mm.			250-500 μ			100-250 μ			25-100 μ					
I*	H†	M‡	I	H	M	I	H	M	I	H	M			
.	mm. Hg		
.	Not taken	Terminal cyanosis	Bronchopneumonia
.	Not taken	Murmur	Dehydration and infection
.	Not taken	Cyanosis on effort	Pneumonia
.	Not taken	None	Hydrocephalus (post-operative)
.	Not taken	Murmur	Meningitis and pneumonia
.	.	.	+	98/70	None	Lead poisoning and medullary compression
.	Not taken	None	Bulbar poliomyelitis
.	Not taken	None	Meningitis
.	+	.	.	95/55	Murmur	Cardiac tamponade and rupture of mycotic aneurysm
.	.	.	+	.	.	.	+	.	.	+	.	118/70	Murmur	Hemopericardium of 1200 cc. following division of ductus
+	.	.	+	.	.	+	++	.	.	++	.	110/56	Murmur	Died during operation
.	+	++	.	+	++	.	142-162 40-0	Murmur	Died following operation
.	+	+	.	.	+	.	105/40	Murmur	S.b.e.
+	+	.	.	+	.	104/54	Murmur; dyspnea, 8 yrs.	Hemorrhage due to silver clip in left pulmonary artery with erosion
.	+	112/60	Murmur	S.b.e. and congestive heart failure
.	.	.	+	.	.	.	+	.	+	++	.	90/30	Murmur	S.b.e. and congestive heart failure; pulmonary embolus

TABLE III (cont'd.)

Autopsy	Age	Sex	Internal diameter of ductus	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
PBBH A-41-127	years 25	M	mm. 4	gm. 400	mm. 7	mm. 15	Healed s.b.e. of ductus	Normal
MGH 10,884	26	F	10	400	4	16	None	Plaque opposite ductus
PBBH A-44-102	27	F	15	560	5	18	Diffuse hypoplasia of aorta distal to ductus	Normal
BCH A-99-85	30	M	4	510	5-8	18	None	Calcification and thrombus in region of ductus
PBBH A-43-102	31	M	Aorta, 5; pulmonary artery, 4	"Moderate hypertrophy"	7	23	S.b.e. of mitral valve and left auricle	Normal
BCH A-43-64	37	M	12	680	18-22	14	Healed pulmonic endocarditis	Marked atherosclerosis of large branches; pulmonary artery, 12 cm. in circumference
BCH A-41-580	44	M	Patent	380	5	18	None	Endarteritis in vicinity of ductus
PBBH A-32-136	47	M	Aorta, 15; pulmonary artery, 5	480	4-6	15-18	S.b.e. of mitral valve and left auricle	Normal
PBBH A-36-173	65	F	3	440	6	17	Calcified annulus fibrosus of mitral and aortic valves [†]	Atherosclerosis of large branches

* Intimal proliferation.

† Hyalin.

‡ Medial thickening.

§ Subacute bacterial endocarditis.

sure was normal. It would appear that there is no constant relation between either the diameter of the ductus or the calculated volume flow and the degree of pulmonary hypertension.

It has been suggested that there is a direct correlation between the size of the ductus, the circumference of the pulmonary artery, and the thickness of the right ventricle. These have been considered to be indices of pulmonary hypertension. In our series there was no correlation between the diameter of the ductus and right ventricular hypertrophy. The right ventricle was of normal thickness in 5 of 8 cases in which the internal diameter of the ductus was 10 mm. or more.

Pulmonary vascular lesions												Blood pressure	Signs and symptoms referable to ductus	Cause of death
Microscopic														
1 mm.			250-500μ			100-250μ			25-100μ					
I*	H†	M‡	I	H	M	I	H	M	I	H	M			
												mm. Hg		
+	.	.	+	.	.	+	108/68	Murmur	Accidental
.	+	.	.	+	.	105/80	Murmur	Died during operation
.	136/50	Murmur; dyspnea, 6 yrs.	Died during operation
.	+	.	—	None recorded	S.b.e.
.	+	++	.	+	++	.	144/72	Murmur	S.b.e.
.	.	.	+	+	.	++++	++++	.	.	+	.	135/85	Murmur	Acute congestive heart failure
.	+	++	.	.	+	.	126/80	None	Portal cirrhosis
+	.	.	+	.	.	++	+	.	++	+	.	120/80	Murmur; dyspnea, 6 mos.	Bacterial endocarditis
.	+	.	.	++	++	.	170/70	Murmur	Unknown; autopsy limited to heart and lungs

Conversely, the right ventricle was hypertrophied in 11 cases, yet 6 of these had defects of 5 mm. or less.

The left ventricle was enlarged in 15 of our 25 cases. The diameter of the ductus was 10 mm. or more in 7 and less than 10 mm. in 8 cases. The heart was increased in weight in 16 of the 23 cases in which the weights were given, and yet in only 5 of these were the ducti over 10 mm. In brief, in this series there was no consistent correlation between the size of the ductus and the degree of left ventricular hypertrophy or increase in weight of the heart.

In patent ductus arteriosus there is a marked increase in blood flow

through the pulmonary circuit. Eppinger, Burwell, and Gross⁷ have measured the increased blood flow occurring in these cases and have found that 45 to 75 per cent of the blood entering the aorta from the left ventricle passes into the pulmonary artery. Subsequent studies have shown that the normal pulmonary blood flow may be increased up to 300 per cent.⁵ The lungs apparently are able to handle this increased volume flow in most cases without any significant elevation of pulmonary arterial pressure until failure of the left ventricle occurs. The reasons for this are manifold. The most important factor probably is the increase in cross sectional area in progressing from the pulmonary artery to the pulmonary capillary bed. This is an approximate increase of from 6 to 38,000 square cm.⁸ In addition, the capillary blood volume can be increased by simple distention of the capillaries.⁹ This occurs at the expense of vital capacity,¹⁰ yet there is no loss of capillary function in the absence of parenchymatous disease of the lung. While this ability to distend is very valuable in the capillary area, it is equally valuable in the larger branches of the pulmonary arterial tree. Morphologic evidence of this is offered by the loose arrangement of the adventitial coat of these vessels. Experimental proof is afforded by the pressure-volume diagrams of Hochrein¹¹ and by studies of pulse wave velocity in the pulmonary artery in comparison to the aorta.¹²

Much has been written concerning structural variations and functional differences in the arterioles of the pulmonary and the systemic circulations. Some writers deny the existence of such vessels in the lungs, others claim that there is a simple decrease in the number of smooth muscle cells in the medial layer, while still others describe variations in diameter of a pulmonary arteriole up to four times that found in the systemic group. In this survey there appeared to be an orderly change in caliber in the branches of the pulmonary arteries and appropriate changes in the cellular structure of the layers in descending to the precapillary level.

However, in approaching this question from the physiologic point of view, Hamilton¹³ has shown a difference of pharmacologic response in the arterioles of the two circulations and that the usual vasomotor response of the arteriole is lacking in the pulmonary system. There is at least one protective reflex mechanism present in the pulmonary vascular bed. The exact receptor area has not been accurately defined, but any increase in intravascular tension results in a significant hypotension of the systemic circulation with an associated bradycardia.¹⁴ This mechanism is demonstrated in experimental pulmonary embolism when precapillary vessels are occluded by *Lycopodium* spores.¹⁵ Here

the pulmonary arterial pressure rises, the femoral arterial pressure falls, and there is a decrease in cardiac output. With this one exception the pulmonary blood flow plays the active rôle and the pulmonary vascular bed a decidedly passive one.

All of the factors mentioned above combine to enable the lungs to care for the increased volume flow when the ductus is patent as long as venous return is unimpeded. In the majority of these cases there is no significant peripheral resistance in the pulmonary vascular bed, and pulmonary hypertension does not develop. Parker and Weiss¹ have postulated three factors that must be present for the production of an abnormal degree of pulmonary arterial and arteriolar sclerosis: High intravascular pressure, stagnation of blood flow, and pericapillary edema. None of these factors is present with a patent ductus until congestive failure supervenes. It is possible that a fourth factor, increase in volume flow, may initiate arteriosclerotic changes in the pulmonary vessels. This would account for the changes in case BCH no. A-43-64 and in the case reported by Keys and Shapiro.⁴ Each of these patients had a large ductus (12 and 15 mm., respectively), and each, presumably, had a large increase in volume flow. In addition, they were in the latter part of the fourth decade of life. As will be discussed later, it is in this age group that the patients with large septal defects, in whom there was a marked increase in volume flow, began to show pulmonary vascular lesions in excess of the control group. Unfortunately, in neither of the above cases was there an opportunity to record the pulmonary arterial pressure or the volume flow. Conversely, in the 4 patients having an elevated pulmonary arterial pressure there was no opportunity to examine the pulmonary vasculature. Individual variations in the vulnerability of the pulmonary vessels to atherosclerotic change may also be a factor in these rare cases of marked pulmonary atherosclerosis. It is conceivable that sufficiently severe pulmonary vascular lesions can produce an increase in peripheral resistance in the pulmonary circuit.

Until complete studies can be made, including accurate measurements of pulmonary arterial pressure and volume flow, followed by an opportunity to examine the pulmonary vascular tree, the causal relationship of pulmonary atherosclerosis to pulmonary hypertension in patent ductus arteriosus cannot be answered.

Interauricular Septal Defect

Twenty-five cases were found in which there were significant unguarded interauricular septal defects. Only those cases were chosen in which the defect was greater than 0.8 cm. in diameter. In 17 cases the

TABLE IV
Interauricular Septal Defects

Autopsy	Age	Sex	Measure- ment of defect	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
CH A-47-23	years 5/12	M	cm. 1.7 x 2.5	gm. 66	mm. 8	mm. 7	None	Dilated 2.5 cm. in diameter
CH A-35-138	9/12	M	2.5	85	5	9	None	Normal
CH A-42-56	1	M	1.5	75	4	8	None	Normal
CH A-30-99	7 1/12	F	2.5 x 2.5	59	6	7	None	Normal
CH A-43-89	9 9/12	F	2.5	140	5	15	R.h.d.; moderate mi- tral stenosis and in- sufficiency	Normal
MGH II, I2I	14	F	1.0 x 2.0	380	5-7	17	R.h.d.; mitral and tri- cuspid stenosis	Normal
PBBH A-46-1	16	F	1.0	460	6	20	Hypertensive heart disease	Normal
BIH A-38-47	17	F	0.9	220	2	8	None	Normal
PBBH A-24-2	29	M	0.8	1000	8	20	Constrictive peri- carditis	Normal
BCH 1923-155	34	F	3.5	470	7	11	S.b.e. of tricuspid, pulmonic, mitral and aortic valves	Normal
BCH 1933-241	34	F	4.0	550	13	15	R.h.d.; tricuspid and mitral stenosis; co- arctation of aorta	Dilated 5 cm.; atherosclerosis
BCH A-44-391	36	M	3.0	760	15	13	S.b.e. of mitral valve	Dilated
BIH A-33-94	47	F	2.0	420	10	15	Mitral thickening	Atherosclerosis
BIH A-36-89	51	M	2.0	640	8	14	R.h.d. and mitral stenosis	Atherosclerosis of large vessels
PBBH A-33-119	53	M	0.8	340	4	20	None	Normal

Pulmonary vascular lesions												Blood pressure	Signs and symptoms referable to cardiac lesion	Cause of death
Microscopic														
1 mm.			250-500μ			100-250μ			25-100μ					
I*	H†	M‡	I	H	M	I	H	M	I	H	M			
.	mm. Hg		
.	Not given	Effort cyanosis; murmur	Pneumonia
.	Not given	Cyanosis for 8 mos.	Congestive heart failure
.	Not given	Intermittent cyanosis	Pneumonia
.	Not given	None	Septicemia
.	+	+	.	+	+	.	92/60	Murmurs of mitral disease	Congestive heart failure
+	+	+	.	.	+++	.	100/70	Transient apical systolic murmur	Congestive heart failure
.	+	.	.	+	.	185/140	Grade I systolic and diastolic murmurs	Glomerulonephritis; uremia
.	+	.	125/75	None	Tuberculous meningitis
.	.	.	+	.	.	++	+	.	++	++	.	120/55	Systolic and diastolic murmurs	Congestive heart failure
+	.	.	.	+	.	++	+++	.	+	+++	.	—	Presystolic and apical systolic murmurs; dyspnea for 6 mos.	Congestive heart failure and pneumonia
+	+	+	.	+	++	.	—	Presystolic and apical systolic murmurs; r.h.d. for 24 years	Congestive heart failure and bronchopneumonia
+	.	.	.	+	.	++	+++	.	+++	++	.	85/65	Intermittent cyanosis; clubbing; murmur	Congestive heart failure and infection
+	.	.	++	.	.	++	++	.	+	+++	.	170/116	Dyspnea; cyanosis; and ascites	Congestive heart failure and pneumonia
+	.	.	+	.	.	+	+	.	++	+++	.	120/70	Dyspnea and cyanosis for 6 days	Congestive heart failure
+	+	++	.	++	+	.	110/70	None	Perforated gastric ulcer

TABLE IV (cont'd.)

Autopsy	Age	Sex	Measure- ment of defect	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
	years		cm.	gm.	mm.	mm.		
MGH 6580	56	M	2.4 x 1.5	675	8	11	R.h.d.; aortic and mi- tral stenosis	Dilated; atheroscle- rosis of smaller branches
PBBH A-47-64	57	M	2.5	600	8	18	None	Dilated (11 cm. in circumference); atherosclerosis of large branches
PBBH A-31-112	58	M	2.0	700	4	16	Hypertensive heart disease	Normal
MGH 9784	59	F	3.0	475	9-11	11-13	R.h.d., and s.b.e. of mitral valve	Normal
BCH A-40-803	60	F	3.0 x 1.5 3.0 x 1.5	495	10	15	None	Normal
PBBH A-36-59	60	M	0.7 x 0.7 0.7 x 0.4	280	4	12	None	Normal
MGH 6776	63	M	0.8	480	4	18	None	Normal
MGH 8298	63	M	0.7	300	3	8	None	Normal
MGH 11,560	70	M	1.0	450	5	15	None	Normal
MGH 6800	76	M	2.0	550	4	21	None	Normal

* Intimal proliferation.
† Hyalin.

‡ Medial thickening.
§ Rheumatic heart disease.

|| Subacute bacterial endocarditis.

defect exceeded 2 cm. There were 13 cases without significant cardiac lesions, 9 cases with some degree of rheumatic involvement of the mitral valve, 2 cases of hypertensive heart disease, and one case with constrictive pericarditis.

In the 13 uncomplicated cases there was a direct relation between the size of the defect, cardiac enlargement, and right ventricular hypertrophy. The heart was increased in weight in 6, and 5 of these had defects greater than 2 cm. Of the 7 hearts of normal weight, 6 had defects of 1 cm. or less. In this same group of uncomplicated cases the right ventricle was hypertrophied in the 4 cases having the larger defects. In only 2 of the 13 cases was the pulmonary artery dilated.

In the 13 uncomplicated cases, the pulmonary vascular lesions were not greater than those in the control group of comparable ages. Three

Pulmonary vascular lesions												Blood pressure	Signs and symptoms referable to cardiac lesion	Cause of death
Microscopic														
1 mm.			250-500 μ			100-250 μ			25-100 μ					
I*	H†	M‡	I	H	M	I	H	M	I	H	M			
												mm. Hg		
++	.	.	++	.	.	+++	+	.	++	+++	.	130/70	Dyspnea and ascites	Congestive heart failure
+	.	.	+	.	.	++	++	.	+	+++	.	132/80	Basal systolic murmur	Carcinoma of bladder; congestive heart failure
+	.	.	+	.	.	+	++	.	+++	++	.	240/110	Hypertension	Congestive heart failure and nephrosclerosis
+	.	.	+	.	.	++	++	.	++++	+++	.	110/80	Dyspnea for 4 years	Infection; pneumonia; congestive heart failure
—	—	—	+	.	.	++	+++	.	++	+++	.	160/110	Dyspnea for 2 months	Congestive heart failure and pneumonia
+	.	.	+	.	.	++	++	.	+	+	.	138/68	Apical systolic murmur	Portal cirrhosis; hemorrhage
+	.	.	++	.	.	++	++	.	+	++	.	148/70	None	Pulmonary embolism; carcinoma of colon
—	—	—	+	.	.	+	++	.	+	++	.	130/80	None	Carbuncle; septicemia
++	.	.	+	.	.	+	+++	.	+++	+++	.	140/80	Precordial systolic murmur	Prostatism
+	+	.	.	.	+	.	170/80	None	Pulmonary embolism

of these patients were in the first decade of life and had no vascular lesions, yet the septal defects were all greater than 2.5 cm. and all had cardiac enlargement with right ventricular hypertrophy. These 3 cases are interesting in regard to the time interval required for the production of pulmonary vascular lesions. Cases with marked pulmonary atherosclerosis have been reported by Wätjen¹⁶ and zur Linden,¹⁷ one in a 6-months-old child and the other in an 11-months-old child. However, both of these had complicating cardiac anomalies. Wätjen's case had an associated interventricular septal defect and transposition of the great vessels, while zur Linden's case had a patent ductus.

One case in this uncomplicated group is of special interest in that accurate measurements of pulmonary arterial pressure and volume flow were made. This was the first opportunity to correlate these measure-

ments with changes in the pulmonary vasculature in a patient having no additional complicating pulmonary or cardiovascular disease. Case PBBH no. A-47-64 was that of a 57-year-old man. There was an interauricular septal defect measuring 2.5 cm. in diameter. The heart weighed 600 gm.; the left ventricle measured 1.8 cm. in thickness, and the right ventricle, 0.8 cm. There was marked dilatation of the right auricle and ventricle. The pressure in the pulmonary artery as measured by means of the venous catheter showed only minimal systolic elevation (35/10 mm. Hg) and the volume flow through the pulmonary artery was 14 liters per minute. The pulmonary artery was dilated to 11 cm. in circumference and there was atherosclerosis of the larger branches. Microscopic examination showed that the pulmonary vascular lesions did not exceed those found in comparable ages in the control group. This patient did not have cyanosis or clubbing. He developed mild congestive failure 1 year before death but this was easily controlled with digitalis. His death was due to carcinoma of the urinary bladder. The remarkable feature in this case was the lack of significant pulmonary vascular lesions even in the presence of a marked increase in the pulmonary blood flow.

In the group of cases complicated by other cardiac lesions, the 9 cases of interauricular septal defect in which there was rheumatic involvement of the mitral valve were of special interest. The aortic valve was stenotic in one case and thickened in another. Ages ranged from 9¾ to 59 years, and each decade was represented except the third and seventh. Every defect was greater than 2 cm., and all of the hearts were significantly enlarged. The right ventricle was hypertrophied in all but one case and preponderantly so in all patients beyond the third decade. Four of these cases had right ventricles measuring more than 10 mm. in thickness, with normal left ventricular measurements. All patients died in terminal congestive failure. Five of the 9 patients in this group had marked dilatation and/or gross atherosclerosis of the pulmonary artery.

Pulmonary vascular lesions were found in excess of the control group and at an earlier age in 8 of the 9 cases, with 2 plus and 3 plus lesions being consistently encountered. The vessels measuring from 25 to 250 μ were most severely involved. No medial lesions were found. Thickening of the capillary basement membranes, described by Parker and Weiss¹ as occurring in mitral stenosis, was not found. VonGlahn and Pappenheimer¹⁸ have described a specific type of arteritis which occurred in the lungs and elsewhere in 10 of 47 cases of rheumatic fever. In the earlier stages there was a subendothelial deposition of fibrin with cellular destruction, while in the later stages

the intima was thickened and vascularized. These lesions were not found in the present group. In short, the pulmonary vascular lesions were those of atherosclerosis and amounted to premature ageing of the vessels.

The most typical case in this group was BCH no. A-44-391, a 36-year-old man with an interauricular septal defect of 3 cm. and rheumatic involvement of the mitral valve. The heart weighed 760 gm.; the right ventricle measured 15 mm., the left ventricle, 13 mm. The pulmonary artery was dilated and pulmonary vascular lesions were pronounced. The patient had intermittent cyanosis and clubbing. This case is similar to Lutembacher's¹⁰ original case, that of a 61-year-old woman with an interauricular septal defect of 4 cm. and mitral stenosis, who had marked dilatation and atherosclerosis of the pulmonary arteries.

It would seem that in patients with interauricular septal defects and superimposed rheumatic mitral valvular disease a mechanical factor is introduced which greatly alters the existing dynamics of blood flow within the heart. Because of the stenosis of the mitral valve, there is an obligatory shunting of blood from the left to the right auricle. This throws a greater burden on the right side of the heart with consequent hypertrophy and dilatation of the right auricle. This in turn results in widening and stretching of the original congenital septal defect. There is an even greater pulmonary blood flow than is found in cases of patent ductus arteriosus, and it is followed by the development of widespread pulmonary vascular sclerosis. Because the vascular lesions vary in severity and distribution from case to case, there is a corresponding variation in the resistance of the pulmonary vascular bed. In an occasional case in which the vascular lesions are severe, there is a true pulmonary vascular block and cor pulmonale will develop. This would account for the case cited above in which there were found a 15 mm. right ventricle, cyanosis, clubbing, and right heart failure. Yet in most cases, because of the extensive pulmonary vascular reserve, the vascular sclerosis is of no clinical significance.

In contrast to the above group with interauricular septal defects and mitral disease, one of the 2 cases complicated by hypertensive heart disease deserves special comment. This was PBBH no. A-46-1, a 16-year-old girl with an interauricular septal defect of 1 cm. and a systemic blood pressure of 185/140 mm. Hg. The heart weighed 460 gm.; the right ventricle measured 6 mm., the left, 20 mm. The pulmonary artery was grossly normal and there were no significant microscopic vascular changes. The pulmonary arterial pressure was determined by

TABLE V
Interventricular Septal Defects

Autopsy	Age	Sex	Measure- ment of defect	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
BCH 1940-860	years 6 mo. fetus	M	cm. 0.4	gm. 4.5	mm. —	mm. —	None	Normal
CH A-45-129	8/12	M	0.4	38	3	9	None	Normal
CH A-34-182	¹ 7/12	M	0.8	97	7	10	None	Normal
CH A-38-136	³ 4/12	F	0.5	120	6	12	Acute bacterial endocarditis	Normal
PBBH A-44-42	5	M	0.5	200	4	12	Acute bacterial endocarditis	Normal
MGH 6531	8	F	0.8 x 0.5	135	3	12	Acute bacterial en- docarditis of tricuspid valve	Normal
CH A-40-62	⁸ 9/12	M	0.4 x 0.4 0.8 x 1.0	206	4	14	Acute bacterial en- docarditis of mitral, pulmonic, and tri- cuspid valves	Normal
BCH 1941-325	14	F	0.5	240	3	10	Acute bacterial en- docarditis of tricuspid valve	Normal
BCH 1940-860	20	F	2	500	8-10	11-15	None	Normal
BCH 1941-74	34	M	1.6	590	6-8	15-18	Acute bacterial en- docarditis of mitral and tricuspid valves	Normal
BCH 1934-657	72	M	0.5	490	9	20	None	Normal

* Intimal proliferation.

† Hyalin.

‡ Medial thickening.

means of the venous catheter and was within normal range. The patient died of glomerulonephritis and uremia.

To date, there is no evidence that significant pulmonary arterial hypertension develops in cases of interauricular septal defects. Dexter and his group⁵ found a normal pulmonary arterial pressure in 3 of 8 patients studied by means of the venous catheter. In 4, elevated

Pulmonary vascular lesions												Blood pressure <i>mm. Hg</i>	Signs and symptoms referable to cardiac lesion	Cause of death
Microscopic														
1 mm.			250-500 μ			100-250 μ			25-100 μ					
I*	H†	M‡	I	H	M	I	H	M	I	H	M			
.	—	—	Maternal death
.	Not given	Murmur; cyanosis with infection	Pneumonia
.	+	.	.	Not given	Murmur; cyanosis with infection	Congestive heart failure and pneumonia
.	95/20	Murmur	Septicemia; <i>Staphylococcus aureus</i>
.	105/25	None	Pneumonia and ulcerative endocarditis
.	+	.	Not given	Murmur	Pneumonia
.	120/70	Murmur	Septicemia; <i>Staphylococcus aureus</i>
.	+	.	.	+	++	.	100/0	Systolic murmur; dyspnea for 1 year	Septic infarction of lung
.	+++	.	.	+++	++	.	120/70	Dyspnea; intermittent cyanosis; murmur	Congestive heart failure
.	++	.	+	++	.	138/65	Not given	Pneumonia
.	.	.	+	.	.	+	++	.	++	+++	.	190/55	None	Uremia

pressures were noted but in each instance there were clinical manifestations of congestive heart failure. In one patient without evidence of failure, the pulmonary arterial pressure was moderately elevated (40/14 mm. Hg). In one of Dexter's patients there was a volume flow of 20 liters per minute through the pulmonary artery.²⁰ This was the greatest volume flow recorded in any patient with a left to right shunt.

In summary, then, the cases having isolated interauricular septal defects without complicating rheumatic valvular disease did not show pulmonary vascular changes greater than those found in the control groups. In contrast, those cases with coexisting interauricular septal defect and mitral stenosis had constant and definite atherosclerotic changes in the pulmonary vessels. Furthermore, these lesions were more severe and appeared at an earlier age than in the control group. From the evidence at hand at the present time, the only additional factor present in this second group of cases appears to be an increase in the left to right shunt and hence an increase in the volume flow through the pulmonary artery.

Interventricular Septal Defect

Eleven cases were found with significant unguarded interventricular septal defects. These ranged from 0.4 to 2 cm. in diameter, although only three were greater than 1 cm. in diameter. The patients ranged in age from a 6-months-old fetus to 72 years. Six had complicating bacterial endocarditis. The heart was increased in weight in 9 of the 11 cases. The right ventricle was increased in thickness in 4, while the left ventricle was increased in only 2 cases.

There was no dilatation or gross evidence of atherosclerosis of the pulmonary arteries in any case in this group. Ten did not have microscopic lesions greater than those found in the control group. One case (BCH no. A-40-860), a 20-year-old woman, had a 2 cm. interventricular septal defect, the largest in this series. The heart weighed 500 gm., the right ventricle measured 10 mm. in thickness, while the left ventricle was not thickened. This patient had no complicating endocarditis or valvular disease. There were 2 plus and 3 plus intimal proliferative and hyaline changes in the pulmonary vessels from 25 to 250 μ in diameter. No lesions were present in the larger branches. The media was not involved.

As yet, pulmonary catheterization studies with measurements of pulmonary flow and pulmonary arterial pressures have been carried out in only 3 cases having uncomplicated interventricular septal defects. In 2 the pulmonary arterial pressure was normal, with pulmonary volume flows of 7.1 to 7.9 liters per minute. In the third patient the pulmonary arterial pressure was elevated (100/49 mm. Hg), yet the volume flow through the pulmonary artery was only 8.6 liters per minute. There was no clinical evidence of congestive heart failure in this case. In one of the 2 cases having a normal pulmonary arterial pressure there was a calculated left to right shunt of 4.5 liters per minute.²⁰

Experiments in animals in which intracardiac fistulae were produced are of interest. Holman and Beck²¹ produced interventricular septal defects up to 3 mm. in dogs. The dogs responded first by an increase in the heart rate and later by an increase in the total mass of circulating blood with return of the heart rate to normal. Protocols of animals that were allowed to live as long as 6 months after operation included studies of the lungs. There was consistent hypertrophy of the right ventricle, yet there was no evidence of pulmonary atherosclerosis in the 10 dogs studied. More recently, Eppinger and Gross²² have produced similar defects in dogs and limited the defects to 0.4 to 0.6 cm. A left to right shunt was found which ranged from 20 to 50 per cent of the left ventricular output. There was a corresponding increase in pulmonary blood flow. In these animals, the output of each ventricle was markedly increased and there was a uniform cardiac hypertrophy. No study of the pulmonary vasculature was made.

Interventricular septal defects seldom exceed 1 cm. in diameter, in contrast to interauricular septal defects which may measure up to 5 cm. The dynamics of pulmonary blood flow in Roger's disease resemble closely those encountered in patent ductus arteriosus because in both conditions the shunt occurs at systemic arterial pressure. Similarly, the protective factors enumerated above for patent ductus are present in patients having interventricular septal defects.

Combined Lesions Giving a Left to Right Shunt

Six cases were found in which there was a combination of lesions giving a left to right shunt. Three cases were included in which a patent ductus was associated with either an interauricular or an interventricular septal defect. In one of these cases (CH no. A-40-69), a child, 16 months of age, the ductus had an internal diameter of 5 mm., the interauricular defect measured 2 cm., and there was no mitral valvular disease. This patient had no gross or microscopic changes in the pulmonary vessels. By contrast, there were pulmonary changes in excess of the control group in each of the other 2 cases. In one of these there was a small ductus with a large interventricular defect, while in the second there was a large ductus and a large interauricular defect.

The combination of interventricular and interauricular septal defects occurred in 3 cases. In the first, that of a 10-months-old child, both defects were small and only 1 plus lesions in the smallest vessels were present. The second case had a small interauricular defect, an enormous interventricular defect, normal valves, and there were

TABLE VI
Combined Lesions

Autopsy	Age	Sex	Measure- ment of defect	Heart weight	Thickness of ventricle		Significant associated cardiac defects	Gross
					Right	Left		
	years		cm.	gm.	mm.	mm.		Interauricular
CH A-40-27	10/12	F	IASD, § 0.5; IV- SD, 1.0	Not given	6	12	None	Atherosclerosis; pulmonary conus
MGH 11,516	22	M	IASD, 1.5; IVSD, 5.0	300	20	22	None	Normal
BCH 1934-324	39	F	IASD, 4.0; IVSD, 0.3	540	9	12	Mitral stenosis	Dilated 4.2 cm.
								Interauricular
CH A-45-42	3/12	F	PDA, ¶ 0.3; IVSD 1.3 x 1.1	Not given	7	7	None	Normal
CH A-40-69	4/12	F	PDA, 0.5; IASD, 2.0 x 2.0	26	7	10	None	Normal
CH A-42-71	10	—	PDA, 2.0; IASD, 2.0	Not given	15	12	None	Normal

* Intimal proliferation.
† Hyalin.

‡ Medial thickening.
§ Interauricular septal defect.

|| Interventricular septal defect.
¶ Patent ductus arteriosus.

marked pulmonary vascular changes. In the third case there was a large interauricular septal defect with associated mitral stenosis and an insignificant interventricular defect. The pulmonary artery was dilated, and here again vascular lesions were in excess of those found in the control group.

Acquired Lesions Producing a Left to Right Shunt

There was no opportunity to study patients who had an acquired left to right shunt. To date, several hundred patients have had a systemic vessel anastomosed to the pulmonary artery to overcome the disordered pulmonary hemodynamics occurring in pulmonary stenosis with or without a coexisting septal defect.²³⁻²⁷ These patients comprise the most important group with acquired left to right shunt. It

Microscopic												Blood pressure	Signs and symptoms referable to cardiac lesion or ductus	Cause of death
1 mm.			250-500 μ			100-250 μ			25-100 μ					
I*	H†	M†	I	H	M	I	H	M	I	H	M			
septal defect with interventricular septal defect												mm. Hg		
.	+	.	.	Not given	Systolic and diastolic murmur	Congestive heart failure and pneumonia
+	.	..	+++	+	.	++	+	.	+	+++	.	110/96	Clubbing; cyanosis for 1 year	Congestive heart failure and pneumonia
.	++	++	.	+++	++	.	—	Systolic and diastolic murmurs; dyspnea for 2 years	Congestive heart failure and pneumonia
septal defect or interventricular septal defect with patent ductus arteriosus														
.	++	+	.	+++	+	.	Not given	Systolic murmur; cyanosis with infection	Pneumonia
.	Not given	None	Pneumonia
.	.	.	+	.	.	++	+	.	+++	+	.	—	Basal systolic and diastolic murmur; effort cyanosis	Generalized peritonitis

was with this group in mind that this study was undertaken in an attempt to predict the long-standing effects of such a procedure on the pulmonary vascular tree.

While the life expectancy following successful operation undoubtedly may be improved, because of the remarkable cardiac reserve of these young patients, the question arises as to whether such an operation will accelerate the development of pulmonary atherosclerosis. This might result in gradual obliteration of the finer radicles of the pulmonary arterial tree and diminution in the volume of blood delivered to the pulmonary capillaries. The present series of cases would seem to be of value in answering this question since the altered dynamics of flow produced by these operations are comparable to those found with patent ductus arteriosus. Judging from the cases in this series, the

pulmonary vascular bed is able to handle a large increase in volume flow for considerable periods of time without the development of significant vascular changes. The fact that this increased volume of blood is delivered at systemic pressure into the pulmonary tree is of little importance since the numerous protective mechanisms indicated above are at work to avoid the development of significant peripheral resistance. Consequently, the systemic pressure is rapidly dissipated and significant elevation of the pulmonary arterial pressure does not occur. There appears to be individual variation in the vulnerability of pulmonary arteries to atherosclerosis. It may be that in the rare case particularly vulnerable to atherosclerosis, changes of such severity may develop that there will be a true vascular block proximal to the capillary bed. Such an occurrence would nullify the effects of the anastomosis.

SUMMARY

The lungs from 67 patients having congenital cardiac anomalies in which there was a left to right shunt were studied to determine the effect of the altered hemodynamics on the pulmonary vascular bed. The lesions were graded according to the degree of intimal proliferative, intimal hyalin, and medial changes found.

Control groups of 10 cases for each of the first 7 decades of life were examined to determine the effects of ageing alone on the pulmonary vessels. The incidence of pulmonary atherosclerosis was found to be remarkably high in this group.

Twenty-five cases of patent ductus arteriosus with significant defects were studied. With one exception the changes in the pulmonary vascular system were no greater than changes in the control group in comparable ages. In all cases the changes present were atherosclerotic in type, and no medial lesions were found.

Twenty-five cases with interauricular septal defects of 0.8 cm. or more were selected. In uncomplicated cases the pulmonary vascular lesions were not greater than in the control group. In 9 cases complicated by rheumatic mitral disease, marked and constant atherosclerosis was found in excess of the control group.

Eleven cases having isolated interventricular septal defects were studied. Most of these had defects of less than 1 cm., and the pulmonary vascular lesions were not greater than those of comparable ages in the control group. In one patient with a 2 cm. defect marked atherosclerosis of the pulmonary artery was observed.

Six cases having a combination of lesions giving a left to right shunt were studied. The degree of pulmonary atherosclerotic change in each case was proportionate to the age and to the magnitude of the shunt.

The common factor in the production of pulmonary vascular lesions in the occasional cases in each of the above groups appeared to be a marked increase in the pulmonary blood flow.

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DESCRIPTION OF PLATES

PLATE 120

FIG. 1. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 1 plus intimal proliferation. Van Gieson-Weigert's elastic tissue stain. $\times 135$.

FIG. 2. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 3 plus intimal proliferation. Van Gieson-Weigert's elastic tissue stain. $\times 180$.

1



2

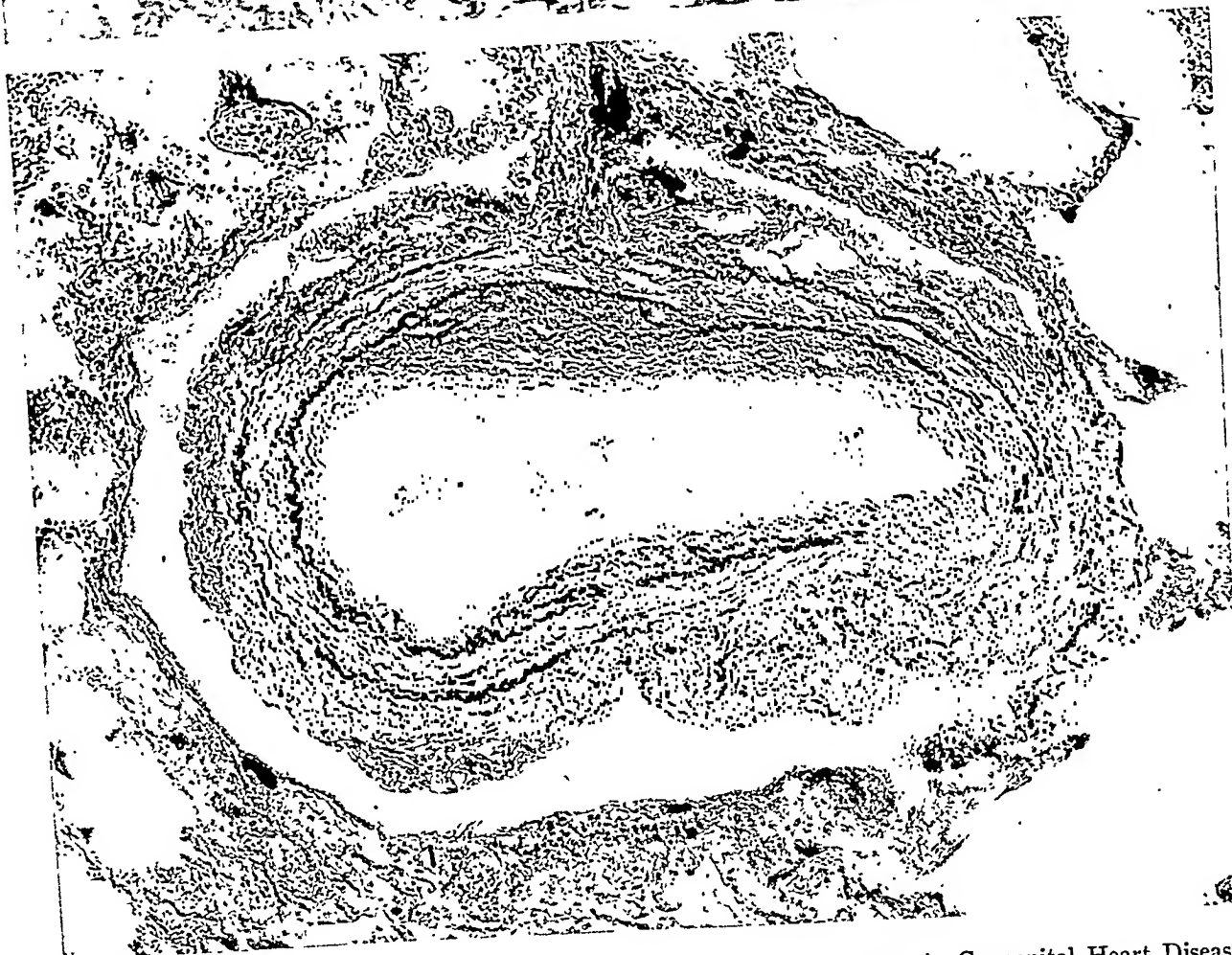
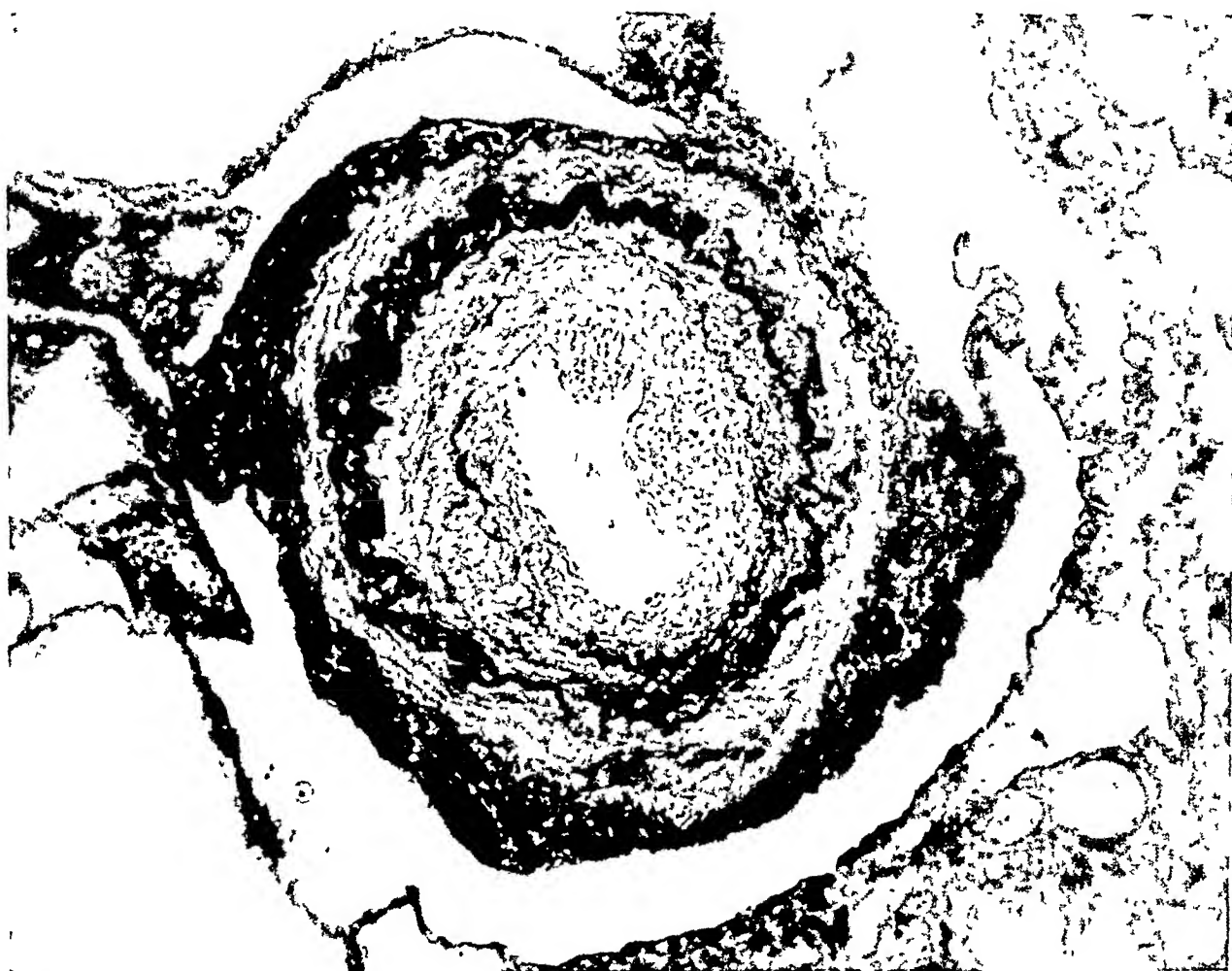


PLATE 121

FIG. 3. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 4 plus intimal proliferation. Van Gieson-Weigert's elastic tissue stain. $\times 225$.

FIG. 4. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 1 plus hyaline deposition. Van Gieson-Weigert's elastic tissue stain. $\times 715$.

3



4

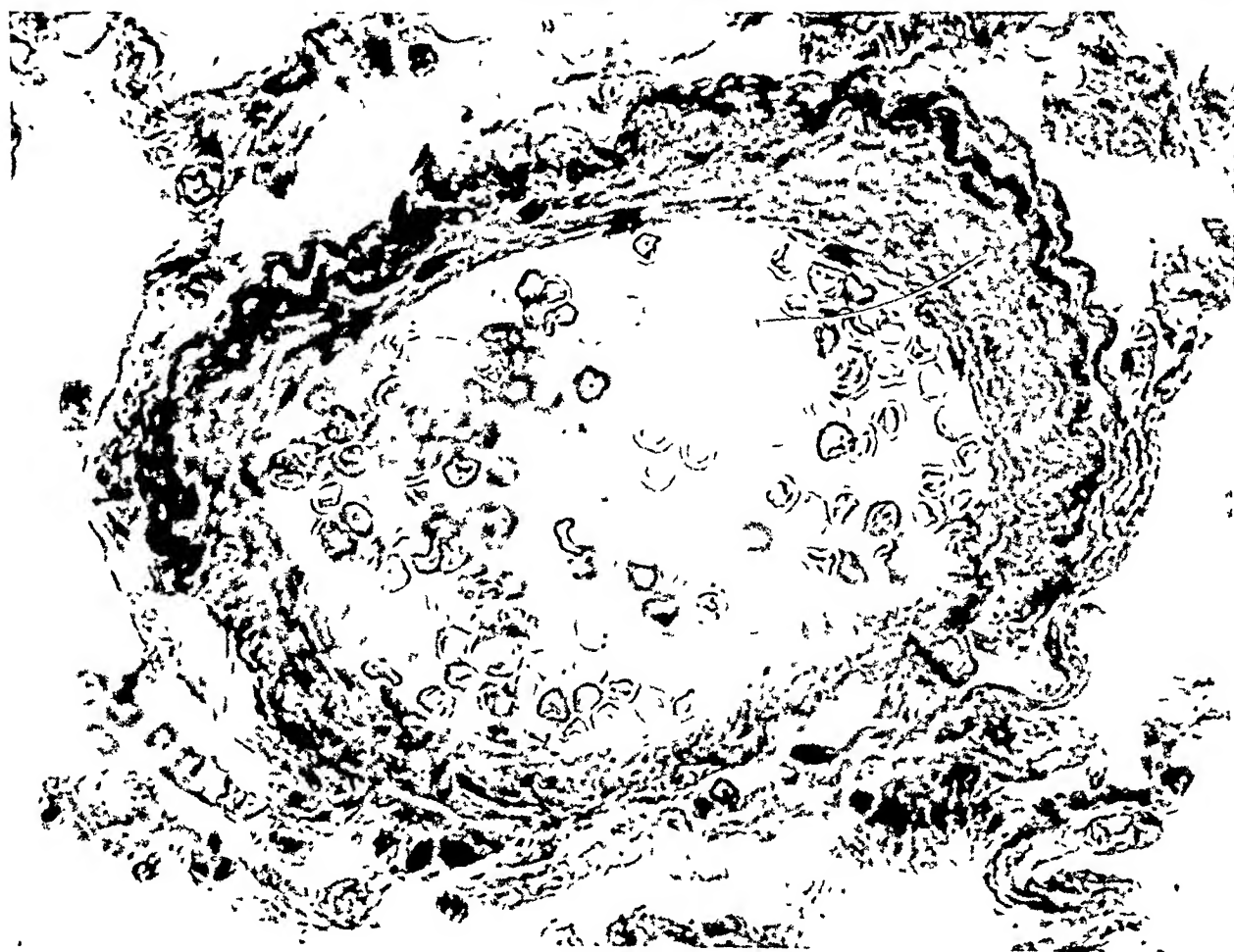
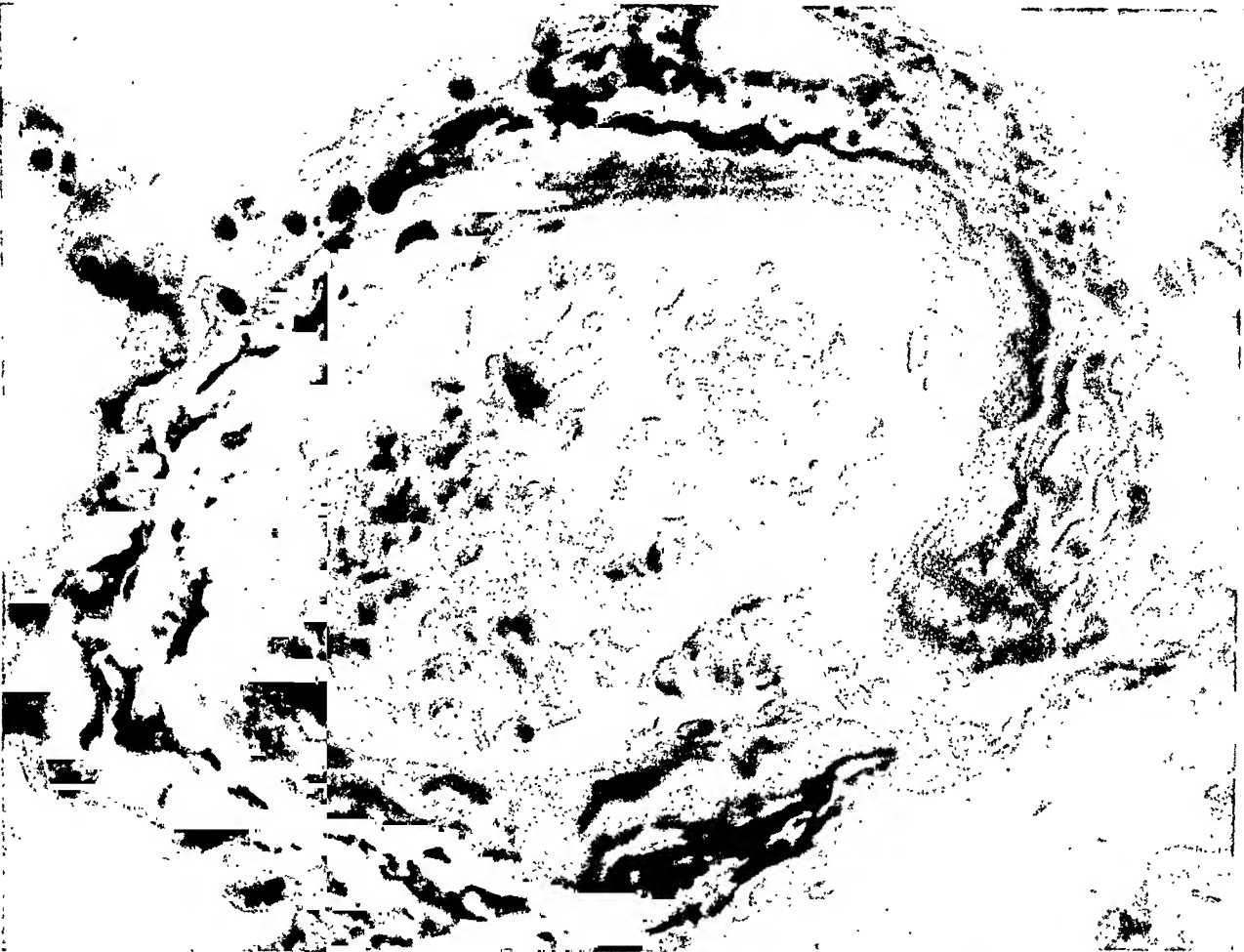


PLATE 122

FIG. 5. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 3 plus hyaline deposition. Van Gieson-Weigert's elastic tissue stain. $\times 580$.

FIG. 6. Pulmonary vessel from the group measuring 100 to 250 μ in external diameter, showing 4 plus hyaline deposition. Van Gieson-Weigert's elastic tissue stain. $\times 1075$.



FULMINATING MENINGOCOCCIC INFECTIONS AND THE SO-CALLED WATERHOUSE-FRIDERICHSEN SYNDROME *

J. HOWARD FERGUSON, M.D., and ORREN D. CHAPMAN, M.D.

(From the Departments of Pathology, and Bacteriology and Parasitology,
Syracuse University College of Medicine, Syracuse, N.Y.)

Whenever the incidence of *Neisseria meningitidis* infections approaches epidemic proportions there is an increase in the fulminating fatal infections, frequently with few or no signs of meningeal involvement. The progress of the disease in these cases is so rapid that death commonly occurs within 12 to 24 hours after the appearance of the first symptom. The presenting clinical picture is frequently one of pharyngitis, fever, and sometimes gastrointestinal symptoms, followed by the rapid development of widespread petechiae, cyanosis, peripheral vascular collapse, and death. This condition has come to be known as the Waterhouse-Friderichsen syndrome, with collapse and sudden death supposedly produced as the result of massive bilateral hemorrhage into the adrenal glands. This syndrome occasionally has been reported as occurring in other types of fulminating bacteriemia, but most frequently is associated with *N. meningitidis* infections.

In the past few years we have had the opportunity of studying 16 cases of acute fulminating meningococcic infection which were autopsied. *N. meningitidis* was recovered from cultures of either blood or cerebrospinal fluid, or both, in each case. The pathologic changes found at autopsy in these cases support the view that the presenting clinical syndrome is the result of an overwhelming bacteriemia and toxemia. It is an accepted fact that massive bilateral adrenal hemorrhage occurs in some cases, but from this series it would appear that these are only associated lesions and are not responsible in themselves for the clinical picture presented. Cases having the same clinical syndrome show no great destruction of adrenal cortical substance.

REPORT OF CASES

Case 1

M. D., a 3-months-old female, entered the hospital because of fever and rapid respiration. She had been apparently well until 12 hours before admission, when she became irritable and rapid breathing was noticed. Fever was noticed about 6 hours later. The past history and family history were negative. Physical examination before admission revealed an acutely ill infant. The temperature was 102° F.; the

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pulse rate, 170; and the respiratory rate, 100. There was no purpura. The right tympanic membrane and the throat were slightly injected. There was slight nuchal rigidity. There was dullness over the right chest with questionably impaired breath sounds and occasional fine râles over both scapular regions. The reflexes were physiologic. When seen in the hospital the infant was in extremis. Over the trunk and extremities was a fine, hemorrhagic, petechial rash. The temperature was 97° F. The child expired $\frac{1}{2}$ hour later.

Summary of Gross Autopsy Findings. Purpuric hemorrhages in skin and serosae; bilateral adrenal hemorrhage; toxic changes in skin and serosae; toxic changes in spleen and kidneys; meninges negative; cultures from blood, cerebrospinal fluid, lung, pericardium, and adrenals were positive for *N. meningitidis*.

Case 2

S. L., a female, 8 years old, was admitted to the hospital because of nausea, diarrhea, and abdominal cramps of 2 days' duration, and delirium and purpura of 12 hours' duration. She had been well previously. During the preceding 2 weeks other children in the family had had "intestinal flu." Two days before admission this patient developed the same symptoms. During the evening, about 12 hours before admission, she appeared feverish and delirious. The following morning she was found to have a purpuric rash over the body and complained of indefinite joint pain and pain in the areas of the rash. On admission she appeared acutely ill, and was irrational and restless. The temperature was 101.6° F.; pulse rate, 122; respiratory rate, 40. Examination revealed mottled purplish areas of subcutaneous hemorrhage over the body, most marked on the legs. These were irregular in shape and size. There were many subconjunctival hemorrhages. There was slight pharyngeal injection and submucosal hemorrhage. There was no nuchal rigidity. The heart and lungs were normal. There was generalized abdominal tenderness and bilateral costovertebral tenderness. Reflexes were physiologic. Examination of the blood showed 15,300 white blood cells with 85 per cent polymorphonuclear cells, of which 63 per cent were nonfilamented. The erythrocytes were normal. Bleeding and clotting times were normal. Six hours after admission she appeared to be much worse. She did not respond to stimuli and the pupils were dilated. Blood pressure could not be obtained; the pulse rate was 220; and temperature, 107.2° F. A lumbar puncture was done and slightly cloudy fluid obtained. Death occurred 12 hours after admission and 24 hours after the onset of acute symptoms.

Summary of Gross Autopsy Findings. Purpuric hemorrhages in skin and mucosae; bilateral otitis media; early acute meningitis. Cultures from cerebral cortex, pericardial and peritoneal fluids, purpuric areas, and both middle ears were positive for *N. meningitidis*. *Diplococcus pneumoniae*, type 4, was recovered from the left middle ear.

Case 3

R. B., a male, 20 years of age, was first seen about 4 p.m., complaining of sore throat. Examination was negative except for evidence of a marked pharyngitis. The patient was seen in the hospital at about 9 p.m. Temperature at this time was 102.5° F. and there were noted a very red throat and a few scattered petechiae in the skin. There were no neurologic signs but, because of the petechiae, a blood culture was taken and a lumbar puncture done. The cerebrospinal fluid showed 130 cells, mostly polymorphonuclear leukocytes. Four grams of sulfadiazine was given

by mouth. The patient rapidly became disoriented and the petechiae increased. About 2 a.m., 10 hours after he was first seen, the patient developed respiratory failure and died.

Summary of Gross Autopsy Findings. Purpura in skin and mucous membranes; bilateral massive hemorrhage in adrenals; acute tracheo-bronchitis; very early meningitis. Cultures of blood and cerebrospinal fluid were positive for *N. meningitidis*.

Case 4

On the day of onset, A. B., a female, 30 years old, had felt well until afternoon when she complained of a slight sore throat. During the evening she had noticed numbness and pain in the arms and legs. There was no headache. She was seen by a physician about 10:30 p.m., when her temperature was 100.4° F.; pulse rate, 96; respiratory rate, 20. Physical examination revealed a few petechiae in the skin. There were no positive neurologic findings. The petechiae increased rapidly. The patient became drowsy and expired about 4 a.m., approximately 12 hours from the onset.

Summary of Gross Autopsy Findings. Purpura in skin and mucous membranes; bilateral hemorrhages in adrenals; toxic changes in spleen and kidneys. Cultures from blood and cerebrospinal fluid were positive for *N. meningitidis*.

Case 5

The patient, J. M., was a 1-year-old female, who was admitted to the hospital because of fever and dyspnea of 12 hours' duration. The past history was negative except for frequent colds. One week before admission she had had a "cold" with coryza and occasional cough. Twelve hours before admission she felt feverish and the cough increased in severity, causing her to vomit four or five times. Dyspnea was noted and she became drowsy. On admission she was acutely ill; respirations were rapid and grunting; there was slight cyanosis. Temperature was 106° F.; respiratory rate, 60. There were fine pinhead-sized petechiae over the neck and on one arm. Physical examination was otherwise not significant. There was no nuchal rigidity; reflexes were physiologic. The child was placed in an oxygen tent. The petechiae rapidly increased, and death occurred 1 hour after admission.

Summary of Gross Autopsy Findings. Purpura of skin and mucous membranes; bilateral hemorrhage in adrenals; acute left otitis media; toxic changes in spleen and kidneys; early meningitis. Blood culture was positive for *N. meningitidis*.

Case 6

J. F., a 22-months-old male, had been seen in a neighboring town by a physician who thought that he had bronchopneumonia. There were numerous purpuric spots over the body and extremities. The mother stated that there had been a few such areas for 2 weeks, but that they had markedly increased in the last few hours. The baby was sent to the hospital but was dead on admission.

Summary of Gross Autopsy Findings. Purpura in skin and mucous membranes; right otitis media; toxic changes in spleen, liver, and kidneys; slight hemorrhage in the adrenals; early meningitis. Cultures of cerebrospinal fluid were positive for *N. meningitidis*.

Case 7

R. W., a male, 33 years of age, was a known chronic alcoholic, employed in a restaurant. He was reported to have been intoxicated daily until the day before death. He was seen sober on that afternoon at about 3 p.m. He said that he was going to bed but did not state any reason. He left a call for 5 a.m., his usual rising hour. He did not answer the call, and later was found dead.

Summary of Gross Autopsy Findings. Purpura in skin and mucous membranes; bilateral hemorrhage in adrenals; toxic changes in liver and kidneys; culture of cerebrospinal fluid yielded *N. meningitidis*; blood culture was contaminated.

Case 8

W. T., a male, 44 years old, was admitted to the hospital in coma. There was an indefinite history of an upper respiratory infection. Otherwise he had been well until the morning of admission. At that time he became sulky and irritable, and later in the day, drowsy and incontinent of urine and feces. He was thought to be intoxicated. At 6 p.m., he was seen by a physician who noted petechiae on the body and nuchal rigidity. He was admitted to the hospital at 9 p.m. His temperature was 103° F.; pulse, 104; respiratory rate, 30; blood pressure, 80/40 mm. Hg. There were generalized petechiae and rigidity of the neck. Physical examination was otherwise negative. Lumbar puncture revealed 3840 cells per cmm. There were many Gram-negative, intracellular diplococci. Cultures of the blood and cerebrospinal fluid revealed *N. meningitidis*. Intravenous sodium sulfadiazine was given, the patient receiving 7.5 gm. in 6½ hours. He became very restless, the pulse was rapid and irregular, and death occurred 8 hours after admission and approximately 22 hours from the onset of the illness.

Summary of Gross Autopsy Findings. Purpura of skin and mucous membranes; moderate meningitis; toxic changes in spleen, liver, and kidneys.

Case 9

D. K. O., a male infant, 1½ years old, was seen by a physician because of fever and petechiae of a few hours' duration. The condition was not recognized and no specific treatment was given. The child was dead when seen several hours later.

Summary of Gross Autopsy Findings. Petechiae in skin, conjunctivae, and serous membranes; early meningitis; no gross change in adrenals. Cultures of cerebrospinal fluid were positive for *N. meningitidis*.

Case 10

The patient, E. O., was a female, 26 years of age, who had had symptoms of a mild cold for 3 days. On the afternoon of admission to the hospital there had developed fever, vomiting, chills, and stupor, followed by convulsions. On admission the patient was stuporous. Petechiae had developed over the entire body. The temperature was 102° F.; pulse, 100; respiratory rate, 46; blood pressure, 140/60 mm. Hg. There was nuchal rigidity and a bilateral Babinski sign. The cerebrospinal fluid was slightly cloudy and contained pus cells and Gram-negative intracellular diplococci resembling *N. meningitidis*. Examination of the blood showed 8,400 white cells, with polymorphonuclear cells, 80 per cent; nonprotein nitrogen, 50 mg. per cent. Blood culture was later reported as positive for *N. meningitidis*. Sodium sulfadiazine was given intravenously and a blood level of

14 mg. per cent established. The patient was given also antimeningococcic serum, adrenal cortical extract intravenously, and parenteral fluid because of low urinary output. Twenty-four hours after admission she became irrational and expired.

Summary of Gross Autopsy Findings. Petechiae of skin and mucous membranes; toxic changes in kidneys; a few small hemorrhages in the adrenals; cirrhosis of the liver. Post-mortem cultures of blood and cerebrospinal fluid were negative.

Case 11

D. K., a boy, 4½ years old, had been well until the evening before admission when he complained of headache, followed by vomiting. In the morning he was irrational and a rash was noted over his body. He was seen by a physician who found, in addition, fever and slight stupor. There was no nuchal rigidity or changes in reflexes. Later he had a convulsion, and was sent to the hospital. On entrance the temperature was 96° F.; pulse, 88; respiratory rate, 28. There was a generalized purpuric macular rash, slight cyanosis, painful rigidity of the neck, hyperactive reflexes, and positive Kernig's sign. Blood pressure was 100/40 mm. Hg. The blood examination showed the hemoglobin to be 14 gm.; red blood cells, 4.64 millions; white blood cells, 23,700; polymorphonuclear cells, 84 per cent. Urine examination revealed no significant change. The cerebrospinal fluid was cloudy, and there were many polymorphonuclear cells and many Gram-negative diplococci resembling meningococci. Cultures of blood and cerebrospinal fluid were later reported as positive for *N. meningitidis*. The patient was given sulfadiazine, adrenal cortical extract, and fluids. That evening, although he appeared somewhat improved, his temperature rose progressively to 105.2° F. and his pulse to 170 and cardiac irregularity developed. The cyanosis increased. The patient was found to be sensitive to serum and desensitization was begun. Some edema of the eyelids developed. This was partially controlled with adrenalin, but as the reaction continued, administration of antimeningococcic serum was stopped. The course was progressively worse, and the patient expired the next morning, 36 hours after admission and 60 hours after the onset of his illness.

Summary of Gross Autopsy Findings. Edema of the eyelids; petechiae in skin and epicardium; meningitis.

Case 12

M. A. was a female, 2½ years old, who was admitted to the hospital with the history of stiff neck, vomiting, and malaise of a few hours' duration. She had had a slight rhinitis for 3 days. The child was acutely ill, with rapid shallow respirations, tachycardia, and a temperature of 106.5° F. There was nuchal rigidity and a positive Kernig's sign. Examination was otherwise negative. Lumbar puncture revealed a clear fluid with no cells. Sugar and chlorides were within normal limits. Cyanosis and dyspnea increased, the temperature rose to 108° F., and the patient died 24 hours after the onset of the acute illness. There was no purpura at any time.

Summary of Gross Autopsy Findings. Pulmonary congestion; congestion of brain; adrenals, negative; blood culture, positive for *N. meningitidis*.

Case 13

E. B., a male, 34 years of age, was admitted to the hospital because of pain in the legs, dyspnea, vomiting, diarrhea of 19 hours' duration, and a purpuric rash of 9 hours' duration. Past history was negative. On examination his temperature

was 100.6° F.; pulse, 100; respiratory rate, 28. There was restlessness and severe dyspnea. A diffuse, blotchy, purpuric rash was present over most of the body. There was no nuchal rigidity. The blood pressure could not be obtained. Neurologic examination was negative. The patient was given adrenal cortical extract intravenously and sulfamerazine orally. The blood pressure was recorded as 90/70 mm. Hg, but fell progressively to 58/45 and later could not be read. The temperature rose progressively to 104.6° F. The patient became very cyanotic and expired 12 hours after admission and 31 hours after the onset of the acute disease. An ante-mortem blood culture was reported as positive for *N. meningitidis*.

Summary of Gross Autopsy Findings. Purpuric rash over entire body; petechiae in conjunctivae and serous membranes; massive hemorrhage in adrenals; cloudy cerebrospinal fluid.

Case 14

A. M. was a male, 53 years old, who had complained of weakness of the lower extremities and nausea 2 hours before admission. His family found him on the floor, unable to talk. A convulsion followed and he was sent to the hospital. On admission his temperature was 99.6° F.; pulse, 84; respiratory rate, 26; blood pressure, 85/65 mm. Hg. The only positive physical findings were slight spasticity and hyperactive reflexes in the upper extremities. The peripheral blood was not remarkable. The urine showed albumin, 2 plus, with a few granular casts and pus cells microscopically. Cerebrospinal fluid pressure was 210 mm. of water. The fluid was cloudy and contained 850 cells per cmm. No organisms were seen in smears. Twenty-one hours after admission petechiae in the skin were noted. The temperature was 102° F. The blood pressure remained around 95/80. The patient became progressively worse and died 28 hours after admission.

Summary of Gross Autopsy Findings. Petechiae on the torso and lower extremities, few in serous membranes; lungs, congested; spleen, soft; no gross hemorrhage in adrenals; meningitis, 2 plus; cerebrospinal fluid positive for *N. meningitidis* by culture.

Case 15

R. M., a 1-year-old male infant, had had a mild upper respiratory infection for 2 days. Approximately 24 hours before admission the child was noted to be restless. This was followed in a few hours by vomiting and a red rash on the skin. The child was sent to the hospital. The temperature was 103° F.; pulse, 165, weak and rapid; respiratory rate, 65; blood pressure, 145/100 mm. Hg. The pharynx was red. There was nuchal rigidity, hyperactive reflexes, and bilaterally positive Kernig's sign. Lumbar puncture showed increased spinal fluid pressure, and a cell count of 3600 per cmm., 60 per cent polymorphonuclear leukocytes and 40 per cent lymphocytes. Chlorides, 680 mg. per 100 cc. Meningococci were found in the fluid. The peripheral blood was not remarkable. The child was treated with penicillin intravenously and intrathecally and given sulfamerazine by mouth. Six hours after admission he was lethargic and semistuporous. Temperature was 104° F.; pulse, 175; respiratory rate, 85; blood pressure, 90/60. There were increasing numbers of petechiae, and extreme cyanosis. Death occurred 8 hours after admission.

Summary of Gross Autopsy Findings. Petechiae over upper body, few in the serous membranes; blood-stained fluid in left pleural cavity;

congestion and edema of lungs; congestion of kidneys; negative adrenals; meningitis.

Case 16

I. L., a boy, 5 years old, was seen by a physician because of nausea and vomiting, fever, and a skin rash of 12 hours' duration. He had had a slight "cold" for several days. On admission to the hospital, the temperature was 103.6° F.; pulse, 200; respiratory rate, 20. The blood pressure could not be obtained. The child was deeply cyanotic and in coma. There were numerous petechiae in the conjunctivae and over the entire body, and erythema of the thighs. There was no nuchal rigidity. The reflexes were absent. Death occurred 1 hour after admission, approximately 13 hours after the acute onset.

TABLE I
Pertinent Clinical Data in 16 Cases of Acute Fulminating Meningitis

Case	Sex	Age	First symptoms	First temp. °F.	Purpura	Blood culture	Spinal fluid culture	Duration to death hours
1	F	3 mos.	Fever; irritability	102°	+++	+	+	12
2	F	8 yrs.	Nausea; diarrhea	101.6°	+++	+	+	24
3	M	20 yrs.	Pharyngitis	102.5°	++++	+	+	10
4	F	30 yrs.	Pharyngitis	100.4°	++++	+	+	12
5	F	1 yr.	Fever; dyspnea	106°	++	+	o	13
6	M	22 mos.	Fever	—	+++	o	+	Few
7	M	33 yrs.	?	—	+++	o	+	Few
8	M	44 yrs.	Pharyngitis	103°	++++	+	+	22
9	M	1½ yrs.	Fever	—	++++	o	+	Few
10	F	26 yrs.	Fever	105°	+++	+	+	24
11	M	4½ yrs.	Headache; vomiting	—	++	+	+	60
12	F	2½ yrs.	Malaise; stiff neck	106.5°	o	+	o	24
13	M	34 yrs.	Dyspnea; vomiting	100.6°	++++	+	o	31
14	M	53 yrs.	Nausea; weakness	99.6°	++	o	+	30
15	M	1 yr.	Vomiting; restlessness	103°	+++	o	+	32
16	M	5 yrs.	Vomiting; fever	103.6°	++++	+	+	13

Summary of Gross Autopsy Findings. Petechiae in the skin, numerous petechiae in peritoneum and intestinal tract; bilateral hemorrhagic adrenals; early meningitis; petechiae in brain and meninges.

A summary of the pertinent clinical data is given in Table I.

PATHOLOGIC FINDINGS

A summary of the microscopic changes is found in Tables II and III.

TABLE II
Gross and Microscopic Changes in 16 Cases of *Acute Fulminating Meningitis*

Case	Brain	Choroid plexus	Heart	Lungs	Spleen	Liver
1	Negative		Some Zenker's degeneration	Congestion; slight hemorrhage; slight thrombosis	Congestion	Cloudy swelling
2	Meningitis, 2+	Congestion	Marked Zenker's degeneration; acute reaction; thrombosis	Congestion; hemorrhage; rare thrombus; bronchitis	Congestion	Congestion
3	Meningitis, 1+	Focal acute reaction; marked thrombosis	Marked patchy acute necrosis with polymorphonuclear reaction	Congestion; hemorrhage; few thrombi; acute bronchitis	Splenic tumor	Congestion; swollen endothelium
4	Negative		Focal acute necrosis with polymorphonuclear reaction; capillary thrombi	Congestion; capillary thrombi	Congestion	Few fine hyaline thrombi
5	Meningitis, 1+		Few small inflammatory foci	Congestion; few capillary thrombi, one in small arteriole	Slight necrosis in germinal centers	Many leukocytes in sinuses
6	Meningitis, 1+		Slight Zenker's degeneration; slight polymorphonuclear reaction	Congestion; hemorrhage; acute bronchitis	Congestion	Negative
7	Negative		Few foci of polymorphonuclear and mononuclear cells	Extensive thrombosis of capillaries; small infarcts; hemorrhage, 4+	Slight splenic tumor	Few necrotic liver cells

8	Meningitis, 2+		Moderate foci of polymorphonuclear and mononuclear cells	Extensive thrombosis of capillaries; acute bronchitis	Slight splenic tumor	Congestion; few necrotic cells
9	Meningitis, 1+	Acute reaction; thrombosis	Slight Zenker's degeneration	Congestion	Slight splenic tumor	Fatty change
10	Negative	Early capillary thrombosis	Slight Zenker's degeneration; slight polymorphonuclear reaction	Congestion; few capillary thrombi	Slight splenic tumor	Cirrhosis; fatty change
11	Meningitis, 4+		Slight Zenker's degeneration	Slight bronchopneumonia	Congestion	Fatty change
12	Meningitis, 1+; thrombosis		Slight polymorphonuclear and mononuclear reaction; one thrombus	Congestion and edema	Necrosis in germinal centers	Area of sinus thrombosis
13	Cloudy spinal fluid		Foci of necrosis and polymorphonuclear reaction	Few capillary thrombi	Slight splenic tumor	Central necrosis
14	Meningitis, 3+	Acute reaction	Marked polymorphonuclear and mononuclear reaction	Marked thrombosis; acute bronchitis	Acute splenic tumor	Thrombosis in sinusoids
15	Meningitis, 3+	Thrombosis and acute reaction	Slight Zenker's degeneration; one thrombus	Congestion	Marked necrosis of germinal centers	Fatty change
16	Meningitis, 1+; few thrombi		Inflammatory foci	Congestion and edema	Necrosis of germinal centers	

TABLE III
*Renal and Adrenal Lesions, Thrombosis, and Gross Hemorrhage in the Adrenals,
 Tabulated for 16 Cases of Acute Fulminating Meningitis*

Case	Kidneys	Adrenals	Summary of thrombosis	Gross adrenal hemorrhage
1	Extensive hyaline thrombosis; capsular hemorrhage	Marked hemorrhage; slight acute reaction in medulla; few thrombi	Lungs, kidneys, adrenals	4+
2	Hyaline degeneration of basement membrane; one capillary thrombus	Acute reaction in medulla; thrombi in border capillaries; very slight hemorrhage	Heart, lungs, kidneys, adrenals	0
3	Marked thrombosis; early tubular degeneration	Marked hemorrhage and necrosis; extensive hyaline thrombosis	Lungs, kidneys, adrenals, pancreas, skin	4+
4	Moderate thrombosis; hyaline tubular degeneration	Necrosis and polymorphonuclear infiltration of medulla; areas of cortical necrosis; congestion and hemorrhage	Heart, lungs, liver, kidneys	4+
5	Few areas of hyaline thickening of basement membrane; few thrombi	Congestion and hemorrhage; polymorphonuclear infiltration of medulla; thrombi in large vessels and cortical capillaries	Adrenals; slight in lungs and kidneys	2+
6	Few questionable thrombi in tufts	Intense acute reaction in medulla; slight hemorrhage; moderate thrombosis	Adrenals; questionable in kidneys	1+

7	Marked capillary thrombosis; early tubular degeneration	Extensive hemorrhage and necrosis; focal acute reaction in surviving areas	Marked in lungs and kidneys	4+
8	Early hyaline thrombi in tufts; débris in tubules	Focal mononuclear and polymorphonuclear cells in medulla; thrombus in one vein; slight hemorrhage	Lungs, kidneys, adrenals	o
9	Slight hyaline degeneration in tufts; fatty degeneration in convoluted tubules	Marked capillary thrombosis; congestion; slight hemorrhage	Choroid plexus, adrenals	o
10	Hyaline degeneration; moderate thrombosis	Slight polymorphonuclear reaction in medulla	Choroid plexus, lungs, kidneys	1+
11	Degeneration of tuft endothelium	Negative	None	o
12	Slight hyaline change in tufts	Necrosis and polymorphonuclear reaction in medulla; cortical necrosis; few thrombi	Brain, heart, liver, adrenals	o
13	Moderate capillary thrombosis; tubular degeneration	Hemorrhage; thrombosis; focal necrosis and polymorphonuclear reaction	Lungs, kidneys, adrenals	4+
14	Congestion	Loose fibrin thrombi in central veins	Lungs, liver, adrenals	o
15	Congestion	Necrosis and polymorphonuclear reaction in medulla; thrombosis and slight hemorrhage	Choroid plexus, heart, adrenals	o
16	Congestion	Marked congestion and hemorrhage	Brain	4+

Skin. Hemorrhage into the skin varied from moderate to marked in all cases except one, in which there was none (Fig. 1). Sections through areas of petechiae were available in only 2 cases. In one, diffuse hemorrhage was seen in the corium, without recognizable changes in the blood vessels. In the other, rather diffuse hemorrhage and one area of capillary thrombosis were seen.

Heart. The hearts were not unusual in size. In 12 of the 16 cases, petechial hemorrhages were found in the epicardium and beneath the endocardium. In the epicardium these tended to involve the distribution of the coronary arteries, but scattered hemorrhages were present, also. The endocardial hemorrhages were irregular in distribution. In 6 cases the myocardium was more flabby than usual. Microscopically, all but 4 cases showed varying degrees of leukocytic reaction, either perivascular or in the areas of degeneration, which was largely polymorphonuclear; but in some instances a mixture of polymorphonuclear neutrophils, eosinophils, and mononuclear cells was present (Figs. 2 and 3). In 5 there were definite areas of necrobiotic change of heart muscle, varying from pyknosis of nuclei to complete necrosis of a number of cardiac muscle cells. In 4 cases thrombi were found in capillaries in the myocardium.

Lungs. Grossly, the lungs were not unusual aside from showing varying degrees of congestion and edema. This was evident also microscopically. Actual hemorrhage into interstitial tissue and alveoli varied from slight to marked in all cases. In 5 there was histologic evidence of acute bronchitis, in one instance the exudate was hemorrhagic. Only 2 cases showed evidence of exudation into the alveoli. Hyaline thrombi in capillaries of the lung were commonly seen in 11 of the 16 cases. In 3 they were noted as being slight, in 5 as being moderate, and in 3 as being extensive in degree. In one instance a hyaline thrombus was found in one of the small arterioles.

Spleen. Changes in the spleen were much more marked in the adults than in the children. All of the children's spleens were of normal size, although one appeared slightly softer than usual. In the adults, 6 of the 7 cases showed the gross and microscopic changes associated with early acute splenic tumor and one appeared normal. In some of the children the germinal centers of the splenic corpuscles appeared hyperplastic, but this was not constant. In 4 there was evidence of necrosis of the germinal centers.

Liver. Gross changes in the liver were not remarkable. Microscopically, no uniform change was found. In one case there was rather extensive central necrosis with mononuclear cell reaction. In several there was a slight degree of fatty metamorphosis of the liver cells.

However, in 3 cases there were found areas of hyaline thrombosis in the sinusoids.

Pancreas. Gross and microscopic examination of the pancreas was negative except for the presence of a few hyaline capillary thrombi in 2 cases; one of these was in a child, the other in an adult.

Kidneys. Gross changes in the kidneys were slight but rather uniform. In all cases there appeared slight pouting of the cut surfaces of the kidney substance. The markings tended to be indistinct. In some the kidney substance had a slightly cooked appearance. Punctate areas of hemorrhage into the mucous membranes of the pelves were common. Microscopically, changes in the kidney tissue were rather marked. The predominant lesion was one of hyaline thrombosis in the capillaries of the glomerular tufts. This was present to some degree in 10 of the 16 cases (Fig. 5). In many sections most of the capillary loops of certain glomeruli were occluded by massive reddish hyaline thrombi. In other instances such hyaline material seemed to be present along the capillary walls without completely occluding the vessel. Where thrombosis was present to a less marked degree it was possible to see the fibrinous nature of the material (Fig. 6). In addition to the thrombi in many of the glomeruli, there were scattered areas of hyaline degeneration, apparently involving the endothelial cells of the capillary loops, without thrombosis being present. Exudation of leukocytes into these involved capillary tufts was not prominent, but occasionally polymorphonuclear cells were seen. The tubules were affected in varying degrees. In some there was simple swelling of the cells of the convoluted tubules; in 2 there was rather extensive fatty degeneration of tubular epithelium; in 5 of these cases the tubules contained relatively large quantities of desquamated epithelium and debris.

Adrenals. Gross changes were present in the adrenals in 9 of the 16 cases. In all 9, edema of the perirenal fat and capsule was noted. The adrenals in the involved cases were moderately increased in size but in general maintained their normal outline. On section, reddish purple discoloration of varying degree was found scattered throughout the gland, in some cases almost completely replacing the normal markings. Occasionally, a rim of normal-appearing cortical tissue could be seen around the periphery of the gland substance.

Microscopically, there was only one case in which no definite change could be found in sections of adrenal gland. This was in the child who survived 60 hours. In all other cases, even though the glands appeared grossly normal, definite and usually rather marked microscopic changes were present. These varied in both type and degree. The simplest change consisted of focal areas of necrosis involving the medullary

portion of the gland with an infiltration of polymorphonuclear and eosinophilic leukocytes. In other adrenals this process of acute necrosis and polymorphonuclear infiltration was so marked as to replace the medullary tissue almost completely (Figs. 7 and 8). Focal areas of necrosis and polymorphonuclear infiltration were seen occasionally in the cortical tissue, but these were not prominent. Congestion and hemorrhage were present in these cases to only a slight degree. Hemorrhage was found mostly in the outer areas of the medulla and extended to the inner layers of the cortex. In cases showing more marked involvement, it was so great as to destroy almost completely all normal markings. Occasionally, in the surviving areas, foci of necrosis of both cortex and medulla could be seen. Thrombosis of the adrenals was seen in 11 cases. It was most prominent in the capillaries between the medulla and the cortex (Figs. 9 and 10). In some instances it was massive and extended along the sinusoids between the cords of cortical tissue. In 2 instances both thrombosis and hemorrhage were so marked that the picture suggested massive infarction of cortical tissue. Thrombosis of the central vein of the adrenals was seen in 2 cases (Fig. 4). These thrombi were similar to those seen in the kidney. Where they were present to a slight degree the fibrin constituent could be readily recognized. In areas where they were present to a more marked degree, they were reddish and hyaline. Many of these thrombi could be missed very easily in examination of sections prepared with the routine hematoxylin and eosin stain. They became very prominent when Masson's trichrome stain was used.

The tubular degeneration of the adrenal cortex described by Thomas,¹ Dietrich,² and others, and recently emphasized by Rich,³ was seen in some cases but was not uniform. It was present to the greatest degree in those cases showing the most marked gross and microscopic changes, and did not explain the circulatory collapse in those cases showing minimal adrenal lesions (Table IV).

Sections of the *lymph nodes, thymus, gastrointestinal tract, diaphragm, gallbladder, urinary bladder, aorta, and thyroid* showed nothing significant except for petechial hemorrhages and an occasional capillary thrombus.

Brain. The brain was examined in 15 of the 16 cases. In 5 there was no gross evidence of meningitis. However, in one of these microscopic examination showed a slight infiltration of leukocytes in the pia arachnoid. Congestion of the pia arachnoid and of the brain substance was rather pronounced. In the 10 cases with gross evidence of exudate into the pia arachnoid, microscopic examination revealed the usual histologic changes of acute leptomeningitis. In the one case

(no. 13) in which the head was not opened, a spinal tap was performed at autopsy and slightly cloudy fluid was removed from the subarachnoid space. Focal hemorrhages into the pia arachnoid were common even in the absence of meningeal exudate. In 2 cases microscopic examination revealed several areas of hyaline capillary thrombosis in vessels in the brain tissue.

Choroid Plexus. Sections of the choroid plexus were available in 6 cases. One showed simple congestion. In 4 there was a rather diffuse but focal inflammatory reaction consisting largely of polymorphonu-

TABLE IV

Degree of Meningitis and Adrenal Changes with Available Blood Pressure Readings

Case	Degree of meningitis	Blood pressure	Degree of adrenal hemorrhage	Tubular degeneration	Survival
		<i>mm. Hg</i>			<i>hours</i>
1	o	—	++++	o	12
2	++	Too low to read	o	++++	24
3	+	—	++++	+	10
4	o	—	++++	++++	12
5	+	—	++	o	13
6	+	—	+	o	Few
7	o	—	++++	++	Few
8	++	80/40	o	++	22
9	+	—	o	+	Few
10	o	140/60	+	+	24
11	++++	100/40	o	+	60
12	+	—	o	o	24
13	++ (fluid)	90/70 to 58/45	++++	o	31
14	++++	85/65	o	++++	30
15	+++	145/100 to 90/60	o	+	32
16	+	Too low to read	++++	o	13

clear leukocytes, and hyaline and fibrin thrombi were present in the capillaries of the plexus (Figs. 11 and 12).

Middle Ears. Acute otitis media was found as an associated lesion in 3 of the children.

BACTERIOLOGIC PROCEDURES

It will be noted that *Neisseria meningitidis* was isolated from either the blood or cerebrospinal fluid, or both, in every case in this series. Such successful results are obtained by careful attention to modern bacteriologic detail. The centrifugated sediment from the cerebrospinal fluid is inoculated upon at least three different media; an entire "chocolate" agar plate is prepared from infusion agar base by the addition of 6 per cent citrated horse's blood and heated at 85° C. for 5 minutes, and an entire fresh blood agar plate prepared from infusion agar base by the addition of 6 per cent citrated horse's blood. The contents (5 cc.) of a dextrose semisolid fermentation tube are then

mixed with the remaining sediment, usually in the original specimen tube. This medium is Difco * phenol red broth base to which 0.5 per cent dextrose and 0.2 per cent agar have been added. The dilution of the sediment under such conditions prevents the inhibition of multiplication of the organisms by the enzymes of the leukocytes present and allows for successful isolation in certain instances in which the ordinary cultural methods fail. It is not unusual to obtain a heavy growth of this organism in such tubes in 18 to 24 hours, while a very sparse growth is obtained on the plates at 48 hours. All cultures are incubated at 35° to 36° C.

The blood cultures are prepared by adding approximately 7 to 10 cc. of blood to 70 cc. of medium contained in a rubber-capped bottle under reduced oxygen tension. The medium is a buffered tryptose phosphate broth (Difco *) containing 0.1 per cent agar, 0.1 per cent sodium citrate, and 0.02 per cent para-amino-benzoic acid. If the patient has been receiving penicillin therapy, penicillinase in adequate amounts is added at the time of collection of the blood. These cultures are incubated at 35° to 36° C. and inspected daily or twice daily for evidence of growth.

DISCUSSION

In the 16 cases of overwhelming meningococcic infection presented, there was considerable variation in both the clinical syndrome and in the post-mortem findings.

Of these 16 cases, 10 were males and 6 were females. The age incidence varied from 3 months to 53 years. Nine of these cases were in children ranging in age from 3 months to 8 years, while 7 were adults ranging from 20 to 53 years of age. Although the picture of overwhelming meningococcemia is one which is recognized by pediatricians, the rather common occurrence of this same condition in adults has not been generally recognized. Moritz and Zamcheck⁴ stated: "The incidence of rapidly fatal meningococcemia appears to be considerably higher in younger than in older soldiers." One of the most striking features of the disease is the rapidity of its progress. Although several of these patients had complained of an ordinary upper respiratory infection for a period of 2 or 3 days, most of them (or their parents) could place the onset of acute illness at some particular hour of the day or night. From this time on, 12 of the 16 cases ended fatally within 24 hours or less. Three patients survived about 30 hours, and one survived approximately 60 hours. This confirms the frequently quoted statement of Herrick⁵: "No other infection so quickly slays." Although the presenting symptoms varied considerably, it was notable

* Difco Laboratories, Detroit, Michigan.

that in adults pharyngitis was a common chief complaint, whereas in children fever, headache, and irritability appeared to be particularly common. In patients who were seen by physicians, cyanosis was marked.

Purpura of the skin and mucous membranes was prominent in all except one case. These hemorrhagic manifestations developed rapidly and could be seen to increase rapidly while the patient was under observation. Increasing cyanosis, delirium, stupor, circulatory collapse with falling blood pressure and death soon followed. Cases of this type have been known as the Waterhouse-Friderichsen syndrome. Martland⁶ found only 107 cases of this syndrome prior to 1943 and added 19. Many have been described since.

In most cases the causative organism has been a meningococcus, but other organisms such as *Diplococcus pneumoniae*, *Streptococcus haemolyticus* (beta), *Haemophilus influenzae*, and others have been reported. In all of our cases the meningococcus was isolated from either the blood or cerebrospinal fluid, or both. This is quite in contrast with the statement of Rucks and Hobson⁷ that "search for the causative agent in the spinal fluid is generally fruitless." The reports of McLean and Caffey,⁸ and others, and more recently of Tompkins⁹ on the recovery of organisms from the purpuric lesions offer another method for the rapid recognition of the inciting factor.

The striking change seen at autopsy, in addition to those in the meninges and adrenals, is the microscopic evidence of diffuse and marked vascular damage with thrombosis in many organs of the body. Thrombi were found in the capillary systems of various organs in all except one case—in the patient who survived for 60 hours. In the more severe areas of involvement almost every vessel, in some organs, was occluded by such masses of hyaline material. Such changes were seen in the heart, lungs, liver, kidneys, adrenals, pancreas, and gastrointestinal tract in varying degrees. Hill and Kinney¹⁰ have recently described the marked vascular damage and thrombosis which they found in the skin, membranes, and other organs. In the present series there was no correlation between the amount or degree of thrombosis in one organ and in another, or between the occurrence of such thrombosis and the presence of meningitis. Extensive thrombosis was found in the organs of some patients in whom changes in the adrenal glands were comparatively slight. In others, massive thrombosis occurred in the kidneys and adrenals, and other organs showed comparatively slight changes. In 7 of these cases there was no gross hemorrhage into the adrenals and in 3 it was slight to moderate. Even in cases in which it was marked, much surviving adrenal cortical tissue was seen when

the glands were examined microscopically. In those cases in which the blood pressure was taken there was no correlation between the degree of adrenal cortical destruction and the level of the blood pressure (Table IV). In those cases in which it was not taken, circulatory collapse was evident. Clinically, and otherwise at autopsy, the cases with and without massive hemorrhage into the adrenals cannot be separated. Boger¹¹ stated: "Clinically, the term 'fulminating meningococcemia' seems preferable to 'Waterhouse-Friderichsen syndrome,' and if the latter has any usefulness it should be restricted to pathological discussions." From these studies its use does not appear justified in pathologic discussions. There is evidence of an intense overwhelming bacterial infection with marked vascular damage resulting in thrombosis, hemorrhage, or both. Such changes were found in varying degree in every organ examined, although, as would be expected, not uniformly in all organs in all cases. In some patients the damage in one organ was more marked than in another. The reason for this is unknown, but such unexplained variations in degree of involvement are not unusual in other infections. However, from the evidence presented, there appears no reason for selecting one organ over another for special attention. It is true that, clinically, the collapse that is seen in patients with fulminating meningococcemia simulates the collapse of acute adrenal cortical insufficiency, and that in some cases of such meningococcemia there is massive but not total destruction of adrenal cortical tissue. However, the occurrence of many similar cases without massive involvement of the adrenal cortex appears to minimize the importance of those changes as the productive cause of the clinical syndrome.

There appears to be no correlation between other described changes in the cortex and the degree of circulatory collapse. It is still possible that exhaustion of the cortical cells may be present without histologic changes being produced. It appears probable that such exhaustion takes place more readily in the presence of overwhelming infection, but at the present time there are no criteria for the recognition of such exhaustion. For these reasons we believe the term "Waterhouse-Friderichsen syndrome" should be discontinued. Either the term "fulminating meningococcic infection" or "fulminating meningococcemia" appears justified. In this series, blood cultures taken either antemortem or post-mortem were positive in 11 of 16 cases. One was contaminated, and one was taken only after rather vigorous treatment had been given (case 15). The term "meningococcic meningitis" does not sufficiently separate the fulminating cases from those commonly

seen, and in this series there was no evidence of meningitis at the time of death in 4 cases.

Some clinicians have attempted to divide cases of fulminating meningococcemia into adrenal and meningeal types.¹² As seen in Table IV, in this series, although occasional cases showed marked change in one location and not the other, there is no constant relationship.

Treatment

Considering the overwhelming infection and toxemia in such cases, the value of treatment is a matter of great interest. The fact that patients presenting the classical picture of overwhelming meningococcic infection and intoxication have survived following prompt recognition and vigorous therapy, stimulates one to advocate the use of all available therapeutic agents in adequate dosage. Sulfadiazine by mouth may be satisfactory in the ordinary case of meningococcic meningitis, with or without bacteriemia, but this drug should be used intravenously, in adequate dosage, if the greatest value is to be obtained in the fulminating type of this infection. It is quite apparent that penicillin should be used in conjunction with sulfonamide therapy and that in the cases showing evidence of meningeal involvement it should be administered intrathecally as well as parenterally in adequate and sustained dosage. The use of antimeningococcic serum has been practically discontinued in the treatment of meningococcic meningitis. It is apparent, however, that in the fulminating case the patient needs assistance in combating the overwhelming infection and toxemia until the defense mechanisms can produce sufficient immune substance to sensitize properly its soluble products. The use of antimeningococcic serum in adequate dosage is definitely indicated in the suspected presence of severe infection and intoxication. Adrenal cortical extract has been used by those who believe that the peripheral vascular collapse is due to adrenal damage. At the present time its use must be considered empiric. In this series, cases 2, 4, 6, 9, and 11 were not recognized clinically. Cases 3, 12, and 13 were recognized or suspected, but not adequately treated. Cases 1, 5, 7, and 16 were seen too late for any treatment to be of possible use, and only 4 patients (cases 8, 10, 14, and 15) were given what appeared to be adequate treatment.

SUMMARY

Sixteen fatal cases of fulminating meningococcic infection were studied in which complete post-mortem and bacteriologic examinations were made.

During epidemics, meningococcic infection of this type is not uncommon, and the condition must be recognized and treated properly and early if the mortality rate is to be diminished.

The findings at autopsy are those of an overwhelming bacterial infection with vascular damage, thrombosis and hemorrhage in many organs. Meningeal involvement may be slight or absent.

The term "Waterhouse-Friderichsen syndrome" should be discontinued as evidence shows the condition to be one of general bacterial toxemia, and the occurrence of massive hemorrhage into the adrenal glands is not necessary to produce the peripheral vascular collapse which is so prominent in these cases.

Miss Anne Moran, Assistant Bacteriologist, Bureau of Laboratories, Syracuse Department of Health, and Miss Winifred Osborne, Research Assistant, Department of Bacteriology and Parasitology, rendered valuable technical assistance in the bacteriologic studies herein reported. Grateful acknowledgment is due Miss Stella Zimmer for the photomicrographs.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 123

FIG. 1. Cutaneous purpura. Lesions vary from punctate areas as on the hand to more massive areas as seen on the legs. Present in all cases except one (no. 12).

FIG. 2. Myocardium, showing a lesion of most extensive form. Arteritis with thrombosis and marked perivascular infiltration of neutrophils, eosinophils, and large mononuclear leukocytes. Hematoxylin and eosin stain. $\times 240$.

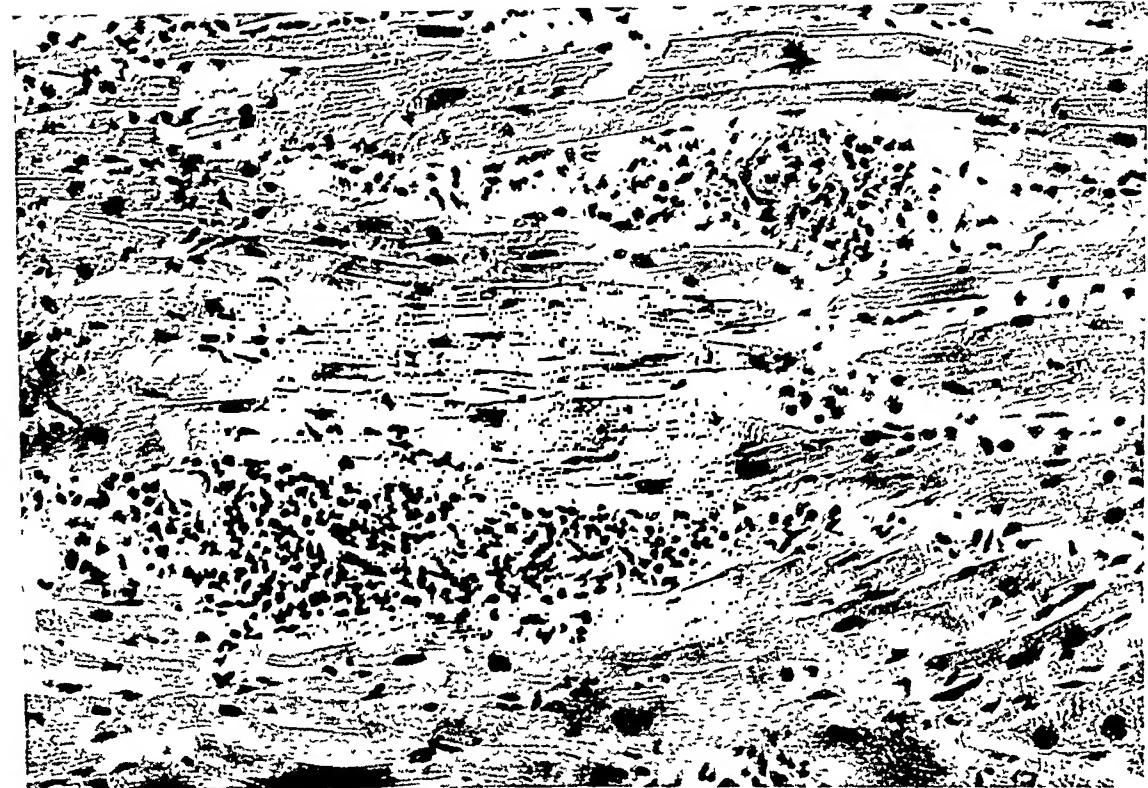
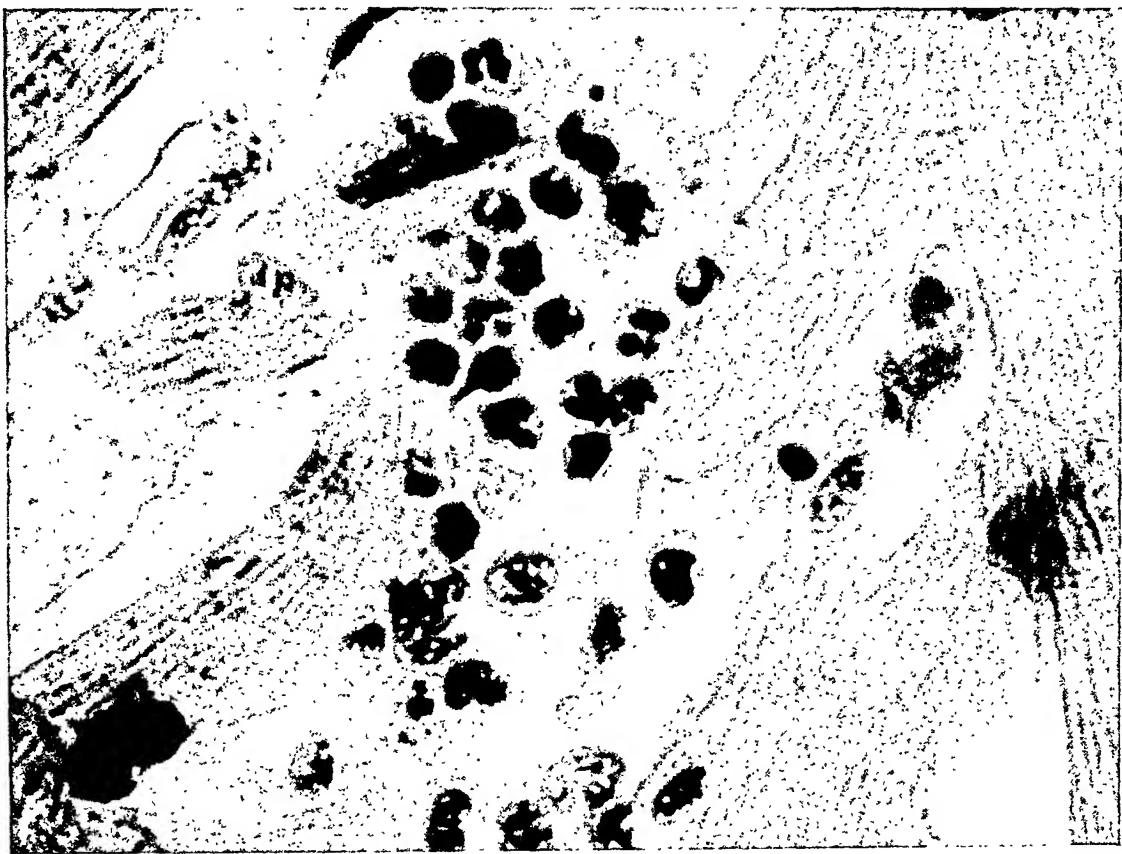


PLATE 124

FIG. 3. Myocardium. Focal area of inflammatory infiltration. Neutrophils, eosinophils, and large mononuclear leukocytes, many with eosinophilic cytoplasm. Myocytes were seen in many fields. Hematoxylin and eosin stain. $\times 950$.

FIG. 4. Adrenal medulla. Mixed thrombus in central vein. Goldner's modification of Masson's trichrome stain. $\times 230$.

3



4

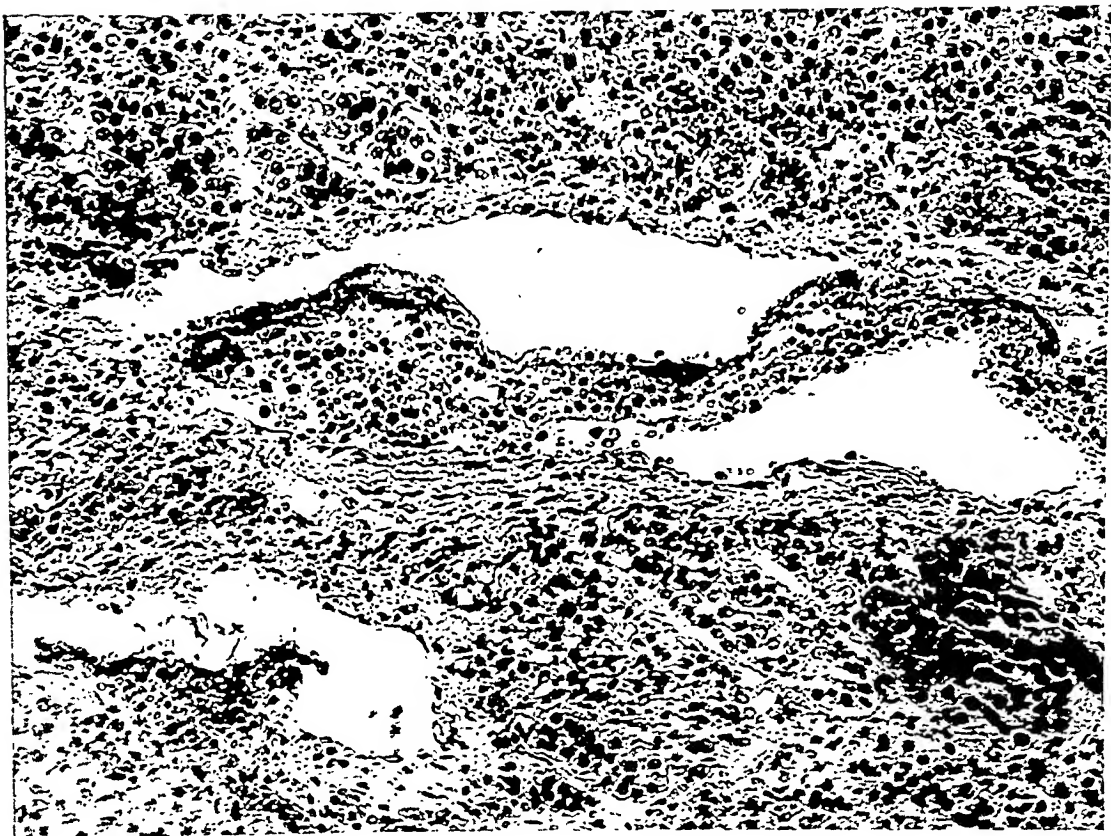
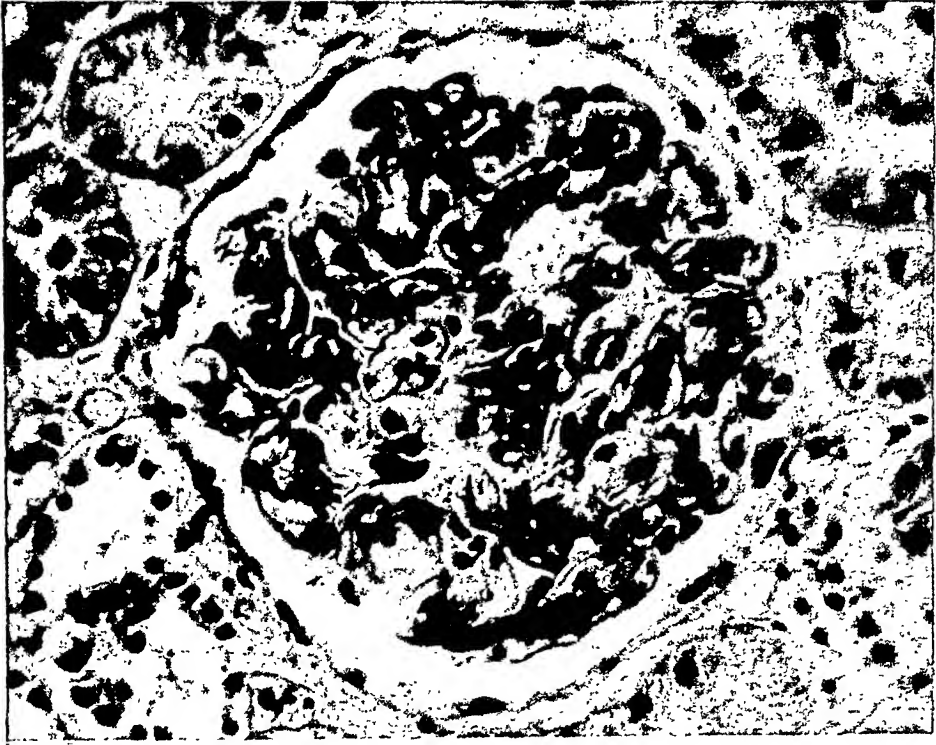


PLATE 125

FIG. 5. Kidney. Marked hyaline thrombosis in glomerular tuft. In some areas the capillary is completely occluded. In other areas the material is attached to the endothelium, leaving a central space. Goldner's modification of Masson's trichrome stain. $\times 420$.

FIG. 6. Higher power of the glomerular tuft seen in Figure 5, showing location and character of hyaline material. Goldner's modification of Masson's trichrome stain. $\times 970$.

5



6

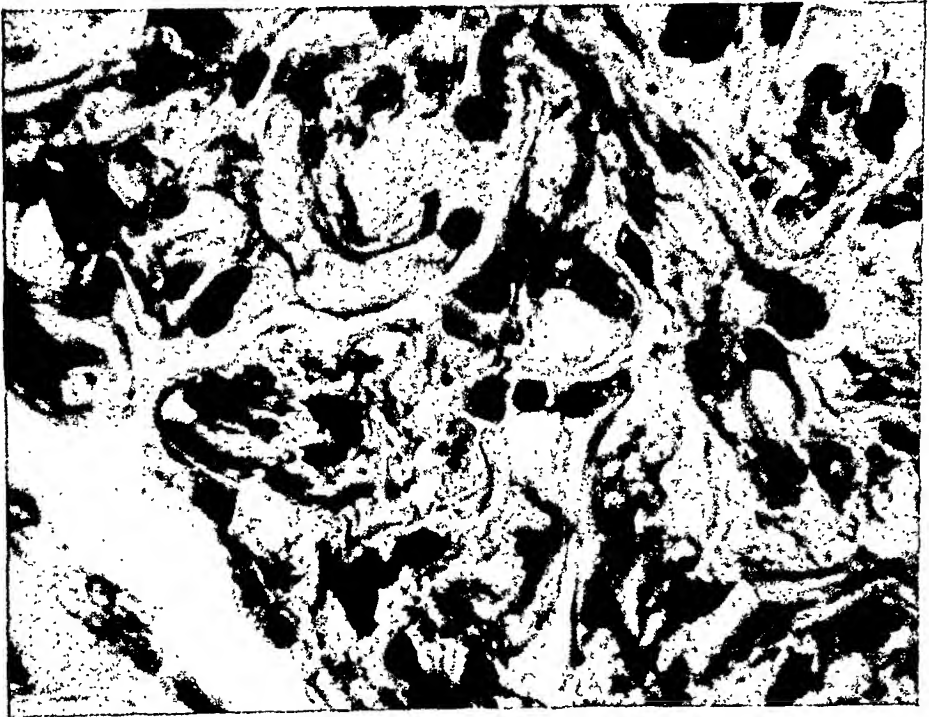
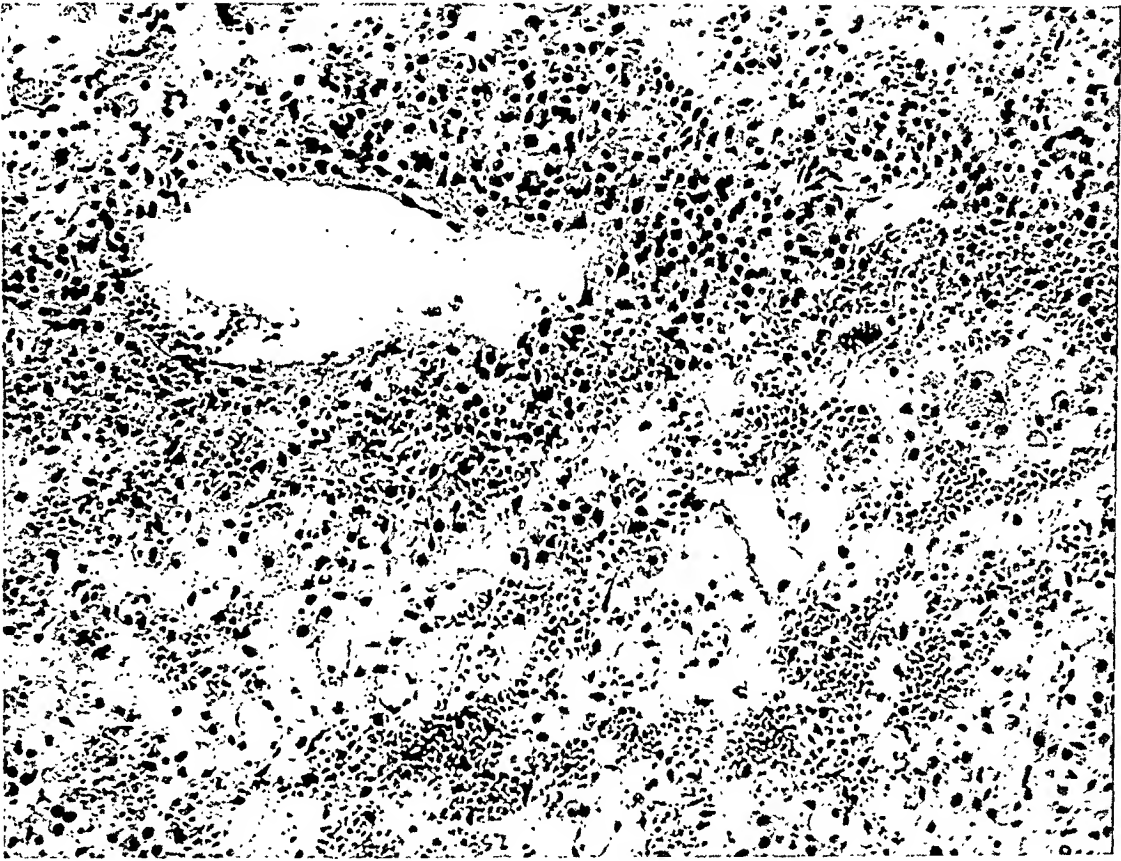


PLATE 126

FIG. 7. Adrenal medulla. Moderate perivascular infiltration of mononuclear cells with some neutrophils and eosinophils. Slight medullary hemorrhage. Hematoxylin and eosin stain. $\times 200$.

FIG. 8. Adrenal medulla. Marked infiltration of neutrophils, eosinophils, and some mononuclear cells. Sparse filamentous fibrin thrombi in sinusoids. Very slight hemorrhage. Hematoxylin and eosin stain. $\times 530$.

7



8

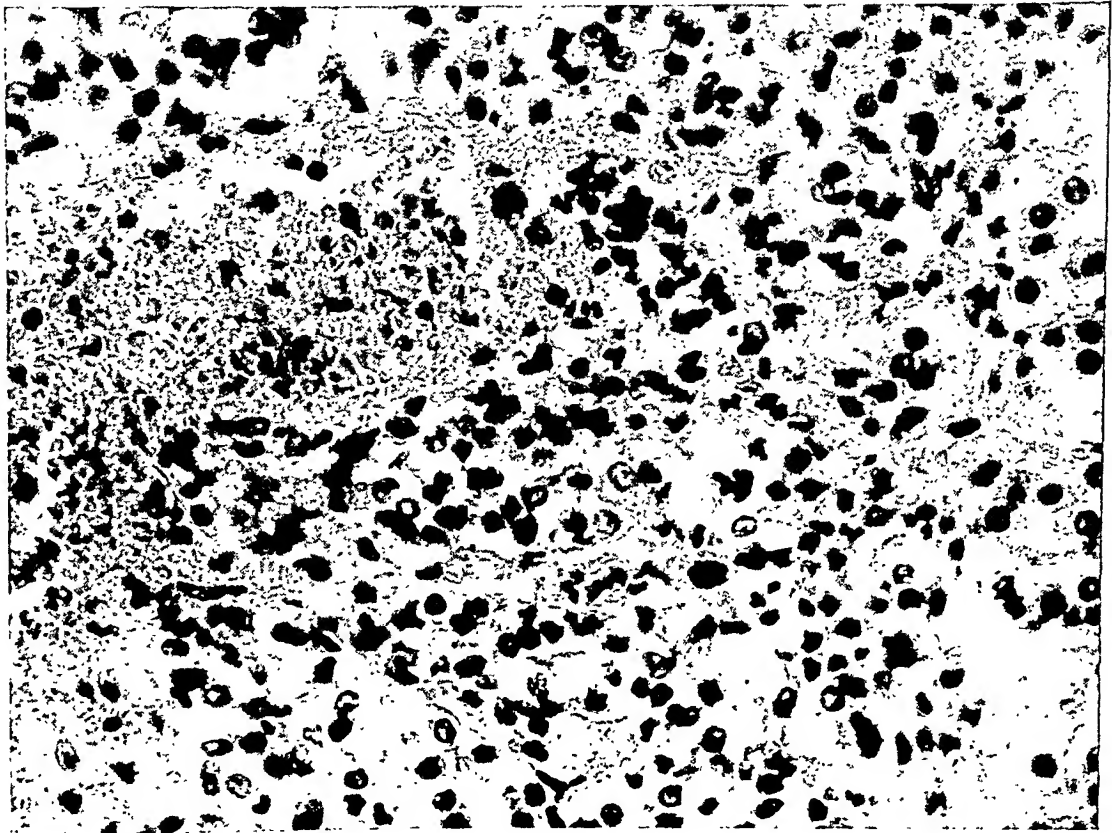
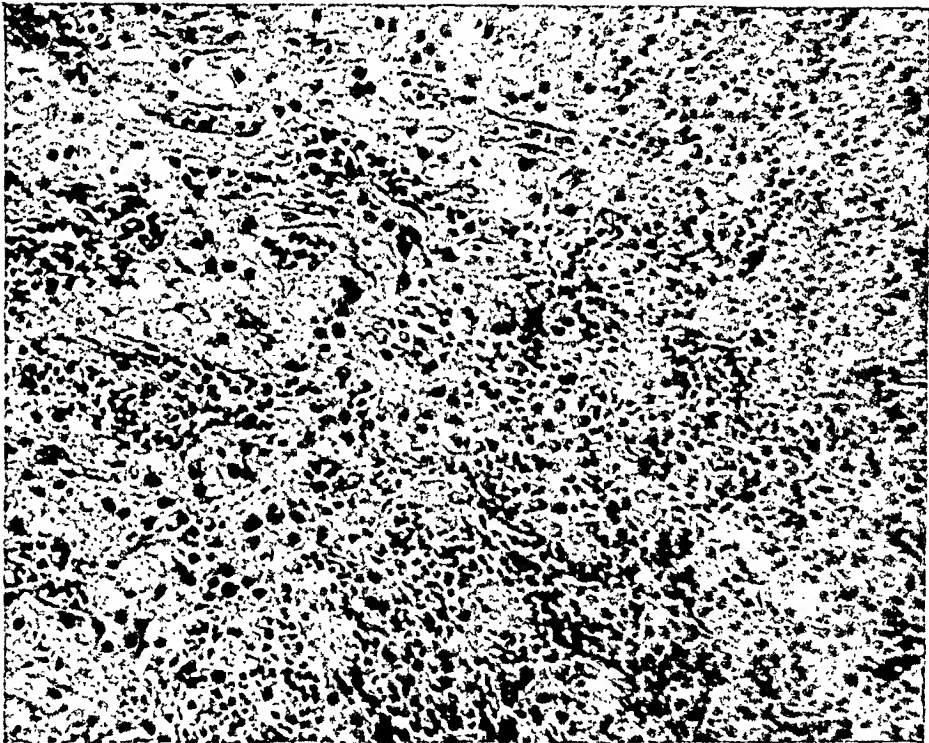


PLATE 127

FIG. 9. Adrenal. Border between medulla and cortex. Rather marked hemorrhage. Most sinusoids are occluded by filamentous hyaline fibrin thrombi. Goldner's modification of Masson's trichrome stain. $\times 230$.

FIG. 10. Adrenal as in Figure 9, showing filamentous hyaline thrombotic material extending along the occluding sinusoids. $\times 950$.

9



10

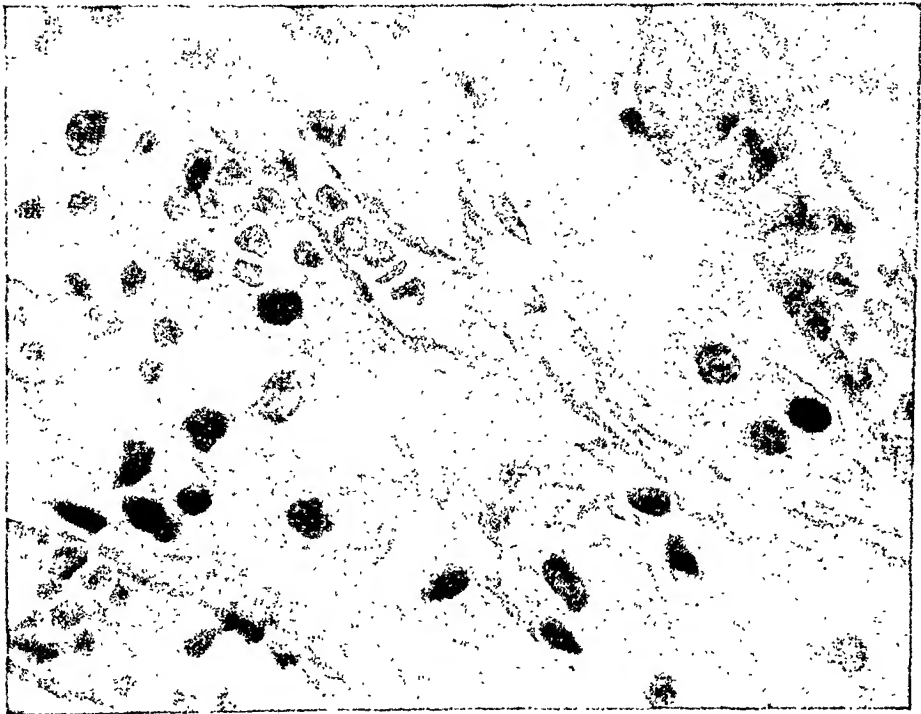
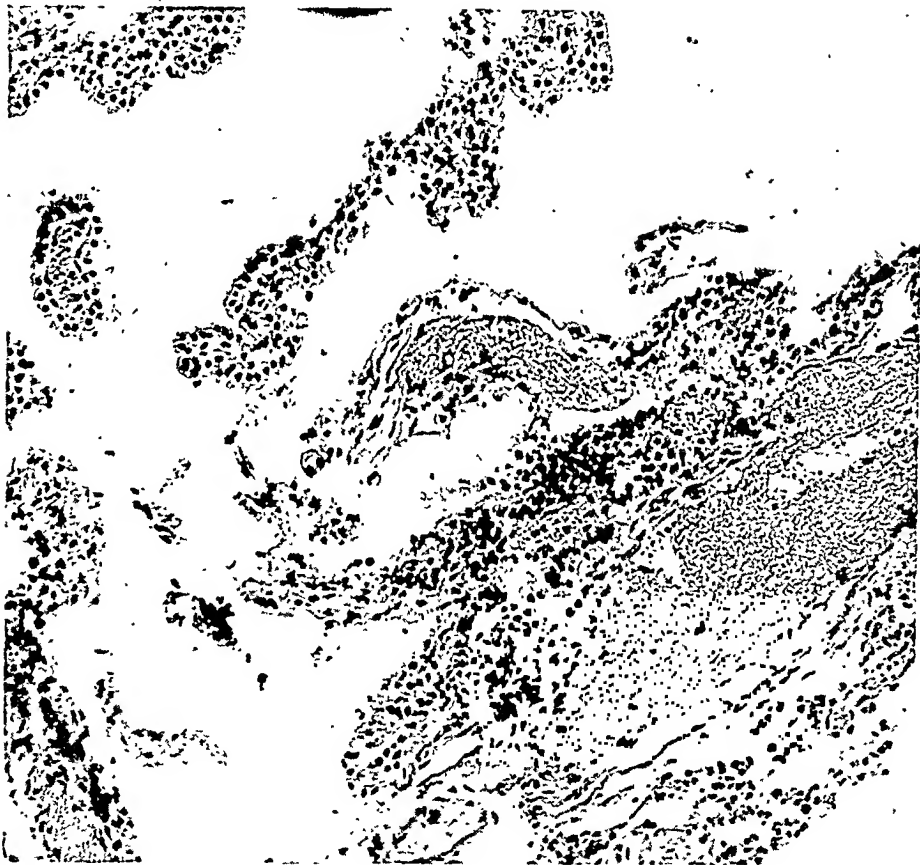


PLATE 128

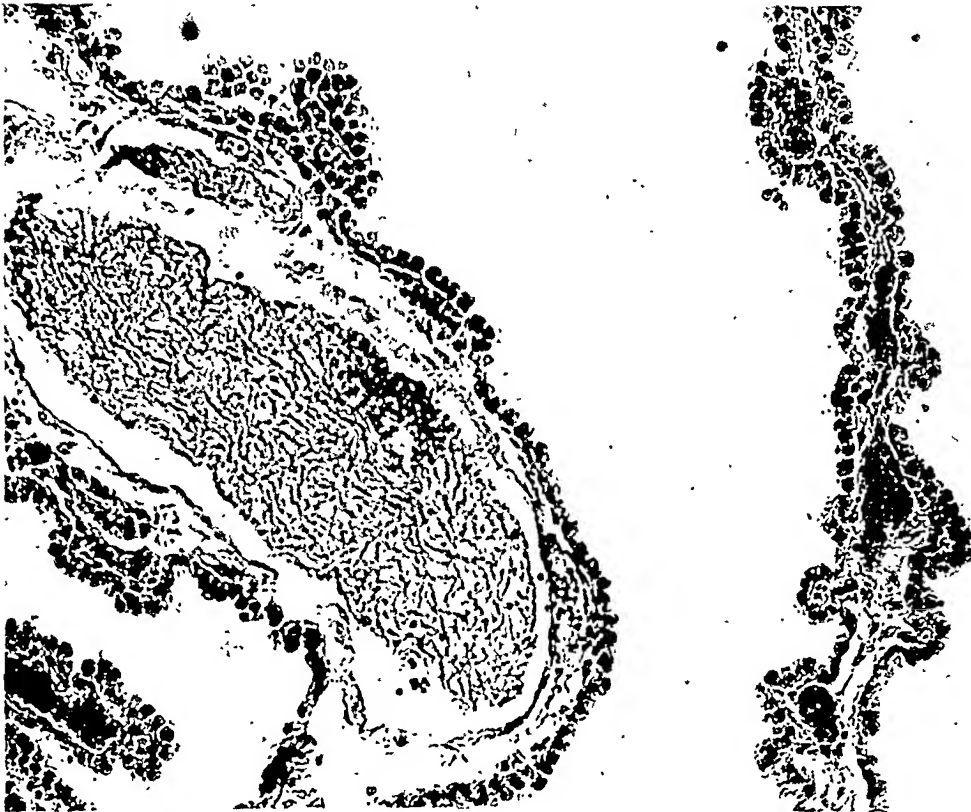
FIG. 11. Choroid plexus. Inflammatory infiltration of interstitial tissue. Thrombosis of veins and sinusoids. Hematoxylin and eosin stain. $\times 115$.

FIG. 12. Choroid plexus. Fibrin thrombus containing few cells. Goldner's modification of Masson's trichrome stain. $\times 115$.

11



12



CYTOLOGIC STUDIES WITH THE PHASE MICROSCOPE
III. ALTERATIONS IN THE NUCLEI OF "RESTING" AND DIVIDING
CELLS INDUCED BY MEANS OF FIXATIVES, ANISOTONIC
SOLUTIONS, ACIDS, AND ALKALI *

HANS U. ZOLLINGER, M.D. †

(From the Department of Pathology of Cornell University Medical College, and the
New York Hospital, New York 21, N.Y.)

Two preceding papers ^{1,2} have dealt with the structure of the cellular membrane and of certain protoplasmic constituents as studied with the phase microscope (PM). The present study is concerned with the nuclei of cells, particularly with the nature of the nucleus as revealed by alterations induced artificially in it.

The method has been described in part I.¹

OBSERVATIONS

The nuclei of normal living cells are spherical, or ovoid, when viewed with the PM. In tumor cells they are mostly irregularly outlined and their shape and size vary. The nuclear membrane is rather thick and black, and shows a few protuberances on its inner side (Fig. 1), whereas the outer side is smooth. The membrane seems to be stretched by the exertion of moderate pressure. Depressions of this membrane, very often observed in microscopic sections, appear in fresh cells only if there are large blisters ¹ in the protoplasm. The chromatin network is loose in fibrocytes and in cylindric cells, and rather dense in liver and kidney cells. The chromatin of malignant tumor cells is usually plump. In all cells there are black, irregular spots or threads of different sizes scattered throughout the nucleoplasm, particularly in the regions of the intersections of the chromatin network. In both normal and tumor cells a slight oscillating movement of these spots and threads is sometimes visible, particularly in the large nuclei of the granulosa cell tumor. These fine granules seem to be stained by Janus green, but their minute size makes this observation very difficult.

The nucleolus is somewhat larger and more regularly outlined than are the black spots of heterochromatin (karyosomes) just described. In kidney cells of the frog it measures about 0.2 to 0.4 μ , whereas the cellular diameter is about 14 μ , and that of the nucleus about 8 μ .

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† Fellow of the Swiss Foundation for Biological-Medical Fellowships.

The degree of shrinkage in paraffin-embedded, and even in frozen sections is evident when these figures are compared with those achieved by measuring kidney cells in microscopic sections, in which the cellular diameter ranges from 8 to 10 μ and the nucleolar diameter from 0.1 to 0.2 μ . The nucleoli of malignant tumor cells are much larger, being 0.3 to 1.4 μ in Brown-Pearce carcinoma cells. In large nuclei (Fig. 2) the nucleoli sometimes move very slightly to and fro. It is not always possible to distinguish between karyosomes and nucleoli unless distilled water is added to the suspension, which causes the disappearance of the chromatin network, leaving the nucleolus clearly visible (Fig. 3).

The nuclear sap, with parts of the chromatin network, flows out, and the cell collapses when a large laceration is made in the nuclear membrane. The nuclear sap disappears in the suspension medium, whereas the nuclear membrane and the rest of the chromatin network shrink considerably. "Naked" nuclei (Fig. 4) often are seen floating in the suspension medium, probably because the membranes of the cells have been destroyed during the preparation of the cell suspension. The "naked" nuclei remain morphologically unchanged in physiologic saline or buffered glucose Ringer solution at room temperature for 5 to 20 minutes.

The nuclei swell considerably and very rapidly when the physiologic medium under the coverslip is replaced by distilled water. The nuclear membranes become very thin and regular, and the nucleoplasm appears hazy and homogenous, whereas the nucleolus remains visible (Fig. 3). Further replacement of the distilled water by physiologic saline solution causes the reappearance of the chromatin network, which is now slightly plumper than in the original cells, although its location and arrangement are the same. The nuclear membrane soon increases in width until it reaches its original thickness. This procedure can be repeated several times, the result always being the same.

Molar NaCl first causes the same enlargement and hazy structure of the nucleus as does the addition of distilled water, but after a few seconds the nucleolus also disappears (Fig. 5). The replacement of the molar NaCl by physiologic saline solution is followed by a slight decrease in nuclear size. First, the nucleus remains hazy; however, soon the nucleolus reappears (Fig. 6), although smaller than it was before. A few seconds later, a small, delicate, dark intranuclear network or ring arises around the nucleolus (Figs. 7 and 8). Optically, this network or ring behaves like the chromatin network, although it never shows the same arrangement in a cell as the chromatin network did at the start of the experiment. Then, the rest of the nucleoplasm

loses its hazy appearance and becomes clear. The nuclear membrane remains much thinner than in normal cells. This whole process can be repeated several times with the same cell.

In 0.05 M ammonia, the nucleus swells considerably, turns hazy, and loses its chromatin structure. Then the nucleolus disappears, and finally the nuclear membrane fades (see Fig. 23 of previous study¹). One-tenth and 0.5 M ammonia act much faster, and they first seem to destroy the nuclear membrane in these concentrations. However, the replacement of the ammonia by physiologic saline solution is followed by the reappearance of the nuclear membrane (Fig. 9), but the chromatin network and the nucleolus remain invisible.

When 0.1 per cent hexylresorcinol S.T. 37* and 0.1 per cent zephiran,† two surface-active compounds, are drawn under the coverslip, the nuclei turn hazy as soon as the compound reaches the cells, swelling and showing the same change as in distilled water. The continued action of zephiran causes the disappearance of the nucleolus, whereas hexylresorcinol produces no nucleolar changes. In NaOH of pH 8.3, the nucleolus turns somewhat hazy while the chromatin and the nucleolus remain visible, but the chromatin appears granular rather than net-like. At pH 10.1, the nucleolus enlarges and the chromatin gradually disappears (Fig. 10). These nuclear changes are irreversible.

In a slightly acid medium (pH 5.8), the nucleolus is the first element to turn brilliant and bluish, followed by the nuclear membrane and the chromatin. These changes take place within 5 to 10 seconds, and, even if the acid medium is replaced by buffered saline solution of pH 7.0 as soon as the nucleolus turns brilliant, they are irreversible. Stronger acid (pH 4.0) causes the same irreversible changes. Two-tenths molar HCl produces a very rapid shrinkage of the protoplasm and the nucleus; the various elements of the latter become brilliant, and the thick membrane, wrinkled and double-contoured. Further replacement of this medium by physiologic saline solution brings about no change. If cells have been first in molar NaCl and then in 0.2 molar HCl, the nuclei do not become brilliant, and the nuclear membranes are no longer visible. The cells appear shiny yellow throughout, but are much smaller than are normal cells which have been exposed to HCl without previous treatment by molar NaCl.

The addition of 10 per cent formalin to a fresh suspension of cells is followed after a few seconds by a very slight shrinkage of the nucleus, which appears first hazy and homogenous. The whole cell then becomes more refractive; the nucleolus and the chromatin network

* Sharp & Dohme, Philadelphia, Pa.

†Winthrop Chemical Co., Inc., New York, N.Y.

turn brilliant bluish. The most impressive change is the irregular thickening and the brilliant appearance of the nuclear membrane, which is now double-contoured (Fig. 11). The changes are produced by 100 per cent formalin, but the shrinkage is much more pronounced. The changes are irreversible in both cases.

Cells that have previously been in distilled water are changed in the same way by formalin: the haziness of the nucleus disappears, the chromatin network reappears (Fig. 12) and turns brilliant as well as the nucleolus and the nuclear membrane. When cells which have been in molar NaCl for several minutes are exposed to 10 per cent formalin, there first appears an intranuclear, rather plump network (Fig. 13), which is somewhat similar to that shown in Figures 7 and 8. Several seconds later, the nucleolus, the nuclear membrane, and this plump network slowly turn brilliant (Fig. 14). In contrast to these findings, 10 per cent formalin does not cause the brilliant appearance of any nuclear element in cells which previously have been in 0.05 to 0.5 M ammonia. Such cells remain in the same state as they were before the formalin was added to the suspension. Acetone, 70 and 95 per cent alcohol, and 5 per cent acetic acid (Fig. 15) alter the cells in exactly the same manner as formalin.

A very peculiar change is produced by 3 per cent potassium bichromate: after 2 or 3 seconds, the nuclear membrane becomes thicker, but not brilliant; the chromatin loses its net-like structure and takes the form of irregular, dark dots scattered through the nucleoplasm (Figs. 16 and 17); the nucleolus becomes very distinctly outlined. This alteration can be called an intermediate stage, because 1 or 2 minutes later the nucleus becomes brilliant. Finally, all of the nuclei shrink and show brilliant elements. In this last stage, the cells are not distinguishable from those treated with alcohol, acetone, acetic acid, and formalin.

The bright extracellular ring in Figure 17 represents an optical phenomenon, which is present in the PM around every spherical body, and which is a consequence of the diffraction of the light by spherical cells. The large size of this ring in Figure 17 indicates that the cell has become spherical, whereas the small size of this ring around normal cells, or its absence, demonstrates that these cells are rather flat.

The Nucleus in Mitosis

Mitotic figures can always be seen in suspensions of malignant tumors. They are particularly numerous in the Brown-Pearce carcinoma, the V2 carcinoma (originating in the Shope papilloma), and the C3H sarcoma (Figs. 2, 18, and 19). In contrast to the somewhat flat

shape of cells in the resting phase, the mitotic cells are almost spherical, in general more refractive, and enlarged. Due to their spherical form, they touch the adjacent cells over a much smaller area than do cells in the resting phase. The slightest current in the suspension medium under the coverslip, therefore, detaches mitotic cells long before the other cells are washed away.

The chromosomes of cells in suspensions are somewhat wider under the PM than in fixed and stained sections. In malignant tumor cells, their arrangement is irregular and the various disturbances as described by Koller³ (stickiness, non-disjunction, clumping, etc.) are readily visible. The mitotic chromosome star is surrounded by an indistinct, bright halo (Fig. 18). Spindles, or even traces of them, such as are seen in fixed and stained sections, can never be observed in fresh cells. The protoplasm of mitotic cells is rather bright and contains numerous black granules, which are uniformly small, whereas the black granules in the surrounding resting cells can be much larger (Fig. 18). In mitotic cells of the Shope papilloma, where they are particularly large, these black granules can be stained specifically with Janus green. A few medium-sized storage granules appear only after the suspension has been at 37°C. for at least 30 minutes.

The chromosomes of mitotic cells disappear within 1 or 2 seconds after distilled water or molar NaCl reaches the cell (Fig. 20). In the prophase and metaphase, the perichromosomal halo develops into a slightly hazy space or vesicle in the center of the cell, and first looks like a raspberry. Later, this vesicle becomes spherical and well defined (Fig. 20). In this phase, the only difference between these central vesicles in mitotic cells and the swollen nuclei in the surrounding nonmitotic cells is the fact that the outlining membrane of the former is definitely thinner (Figs. 20 and 21) than that of the latter (Figs. 3 and 5). In the anaphase and the telophase, instead of the single central vesicle, there appear two vesicles separated either by a thin membrane or by two membranes enclosing a small layer of protoplasm. The chromosome star reappears, surrounded by a bright, irregular halo, when distilled water or molar NaCl is replaced by physiologic saline solution (Fig. 22). At the same time, the thin membrane of the central vesicle, or vesicles, shrinks and forms the outline of the irregular halo. The chromosomes show exactly the same arrangement as they did before the experiment. They even show the same structure after the action of distilled water, whereas they are much larger and paler after the cells have been in molar NaCl. This procedure can be repeated several times with the same cells, the result being always the same; *i.e.*, these changes are completely reversible after molar NaCl.

The small black granules in the protoplasm of mitotic cells enlarge slightly in distilled water (Fig. 20), whereas they decrease in molar NaCl. The brilliant storage granules are not noticeably changed. A solution of 0.05 to 0.5 M ammonia brings about a very sudden disappearance of the chromosomes, which do not reappear after the replacement of the ammonia by physiologic saline solution. Various fixatives (alcohol, acetone, formalin, etc.) convert the chromosomes into small, brilliant, bluish hooks. These changes, in contrast to the changes caused by distilled water, or molar saline solution, are irreversible.

DISCUSSION

Viewed with the PM, the nucleus consists of a rather thick, black membrane, a well defined black nucleolus, a chromatin network, and fluid sap. The heterochromatin condensations (karyosomes) and the nucleoli may exhibit brownian movement. The nucleoli are enlarged in malignant tumor cells.

There occur two different types of nuclear reaction to various changes of the suspension medium. The *brilliant type* is produced by formalin (10 and 100 per cent), alcohol, acetone, acetic acid (5 per cent), and by hydrochloric acid. The thickening and the brilliant appearance of the cellular membrane caused by some fixatives had already been observed by Strangeways and Canti,⁴ who used darkfield illumination, and referred to it as a "definite refractile membrane." M. Lewis,⁵ investigating the action of acids and alkali on tissue cultures, succeeded in transforming the brilliant nuclei into normal appearing cells by washing them in physiologic saline solution. However, restoration to the previous nuclear picture, after the brilliant type of change has taken place, was never observed in the experiments reported above.

Under normal conditions, the nuclear membrane, the nucleolus, and, to a lesser degree, the chromatin network of living cells seem to be a colloid in gel-form, fixed like a jelly on a framework of submicroscopic fibrils. The chemicals mentioned above cause the coagulation or precipitation of the colloid (Baker⁶), with an increase in refractivity and definition. The fact that formalin no longer produces the brilliant type of nucleus, if the cell has previously been in 0.5 M ammonia, suggests the dissolution or chemical transformation of this nuclear colloid by 0.5 M ammonia. The nuclear material, which becomes brilliant in formalin, etc., is not dissolved by molar saline solution, since the addition of formalin to cells which have previously been in molar saline solution still produces the brilliant type of nucleus.

The *intermediate stage* of nuclei, caused by 3 per cent potassium bichromate, can be interpreted as an early phase of precipitation, in

which the precipitated particles are still separated by fluid. Later on, the precipitates agglomerate, shrink, and press the fluid out of their meshes.

The *hazy type* of nuclei is produced by the action of distilled water, molar NaCl, ammonia, NaOH (with a pH higher than 8.3), and two surface-active compounds. The fact that the hazy type is completely reversible in respect to distilled water indicates that distilled water does not dissolve any nuclear compound. It seems likely that the distilled water transforms the chromatin by imbibition into a homogenous jelly.

In contrast to the action of distilled water, molar saline solution dissolves part of the chromatin and nuclear membrane, because these two elements never again show their original structure when molar NaCl is replaced by physiologic saline solution. The substance extracted by molar saline solution is precipitated by physiologic saline solution outside of the cells, and thus becomes visible under the PM in the form of very thin, slightly curled fibrils, arranged in loose bundles (Fig. 23). This observation corresponds with those made by Mirsky.⁷ Hoerr⁸ and Mirsky and Pollister⁹ also observed the appearance of a definitely altered network in cells which first had been in molar NaCl, and then in physiologic saline solution. They assumed that this is due to the dissolution of desoxyribonucleoprotein. More recent investigations by Mirsky and Pollister¹⁰ support this opinion. The material, determined by them to be desoxyribonucleoprotein seems, therefore, to be located in the chromatin network and the nuclear membrane. Its dissolution on the one hand, and the sol-formation of the remaining chromatin on the other, are apparently the reason for the hazy type of nuclei in molar NaCl.

The disappearance of the nucleolus in molar NaCl could indicate that it is made of a material which is dissolved by molar NaCl (desoxyribonucleoprotein), but other compounds must also be important nucleolar constituents; otherwise, the nucleolus would not partly reappear in the same location after the substitution of the molar NaCl by physiologic saline solution.

The facts that the nucleolus usually is not stained by the Feulgen stain, and that it disappears under the influence of ribonuclease, led Mirsky⁷ to the assumption that ribonucleic acid constitutes part of the nucleolus. This assumption is shared by Baker,⁶ Thomas,¹¹ and Darlington.¹² Koller,³ however, concluded from the occasionally positive Feulgen reaction that desoxyribonucleic acid must be present in the nucleolus. Koller and Darlington assumed histone to be another constituent of the nucleolus.

Since molar NaCl is said to dissolve desoxyribonucleoprotein, it is

conceivable that this dissolution is the reason for the decrease in nucleolar size seen after the replacement of molar NaCl by physiologic saline solution. The total disappearance of the nucleolus in molar NaCl is probably due to the conversion of the remains into a jelly, the latter process being reversible.

The irreversible changes of the nucleus caused by ammonia and other alkalis are the result of a dissolution of the heterochromatin and of the nucleolus. The two surface-active compounds tested in these experiments seem to alter the protective cellular and nuclear membranes because of their "extraordinarily intrinsic affinity for proteins" (Valko¹³), thus exposing the nucleoplasm to possibly existing slight differences in the pH and osmotic pressure between protoplasm and nucleoplasm (see below).

Generally speaking, acidic solutions, as well as alcohol, acetone, and formalin, cause the nuclear proteins to coagulate, whereas strong alkalis dissolve them. Besides the pH concentration, the specific ionic qualities of the different compounds also play an important rôle (Duryee¹⁴).

Mitotic figures are readily visible with the PM, but the chromosomes appear slightly thicker than in stained sections. They are surrounded by a bright halo, which enlarges greatly when distilled water reaches the cells. Such cells show exactly the same structure as do resting cells in distilled water, in that there is a large, distinctly outlined, hazy "vesicle" in the center. This observation suggests that the nuclear membrane is not fully dissolved during the mitotic process. It is conceivable that the collapse of the nuclear membrane and its decrease in thickness during mitosis are due to a migration of a material from the nuclear membrane to the invisible chromosome threads, which are coated with, and made visible by, this material. The fact that the chromosomes reappear when the molar saline solution is replaced by physiologic saline solution, although somewhat paler than they were before the experiment, indicates that only part of their constituents (desoxyribonucleoprotein ?) is dissolved by molar NaCl.

The numerous fine-grained elements in the protoplasm of mitotic cells are considered by Opie¹⁵ as probably identical to microsomes. The fact that these particles seem to be stained by Janus green would rather indicate that they represent shrunken mitochondria. However, this assumption is not at too great a variance with Opie's opinion, because the microsomes can be considered precursors of the mitochondria.²

The enlargement of mitotic cells is probably due to an increase in osmotic pressure of the protoplasm during mitosis. The mitochondria and the storage granules shrink as a result of this hypertonicity.²

SUMMARY

The structure of resting and dividing nuclei in cells of various types has been studied under various experimental conditions.

Cells exposed to formalin, acids, alcohol, and acetone show a *brilliant type* of nucleus, *i.e.*, their nuclei are irreversibly shrunken, and the nuclear membranes, the nucleoli, and the chromatin network are brilliant, bluish, and double-contoured. Before becoming brilliant, the nuclei of cells in 3 per cent potassium bichromate exhibit an *intermediate* change: the nucleus contains numerous large, irregular dots, and its membrane is slightly thickened, but no nuclear element is brilliant.

Alkali, molar NaCl, and distilled water produce a *hazy type* of nucleus: the nucleoplasm becomes homogenous and hazy gray. This alteration is reversible after the action of distilled water, partly reversible after molar NaCl, and irreversible after ammonia.

The nuclear membrane does not seem to be dissolved during mitosis. The very small, black granules in mitotic cells are considered to be shrunken mitochondria.

These findings concerning the chemical and physical structure of the nuclear elements have certain implications in relation to the conclusions of other authors.

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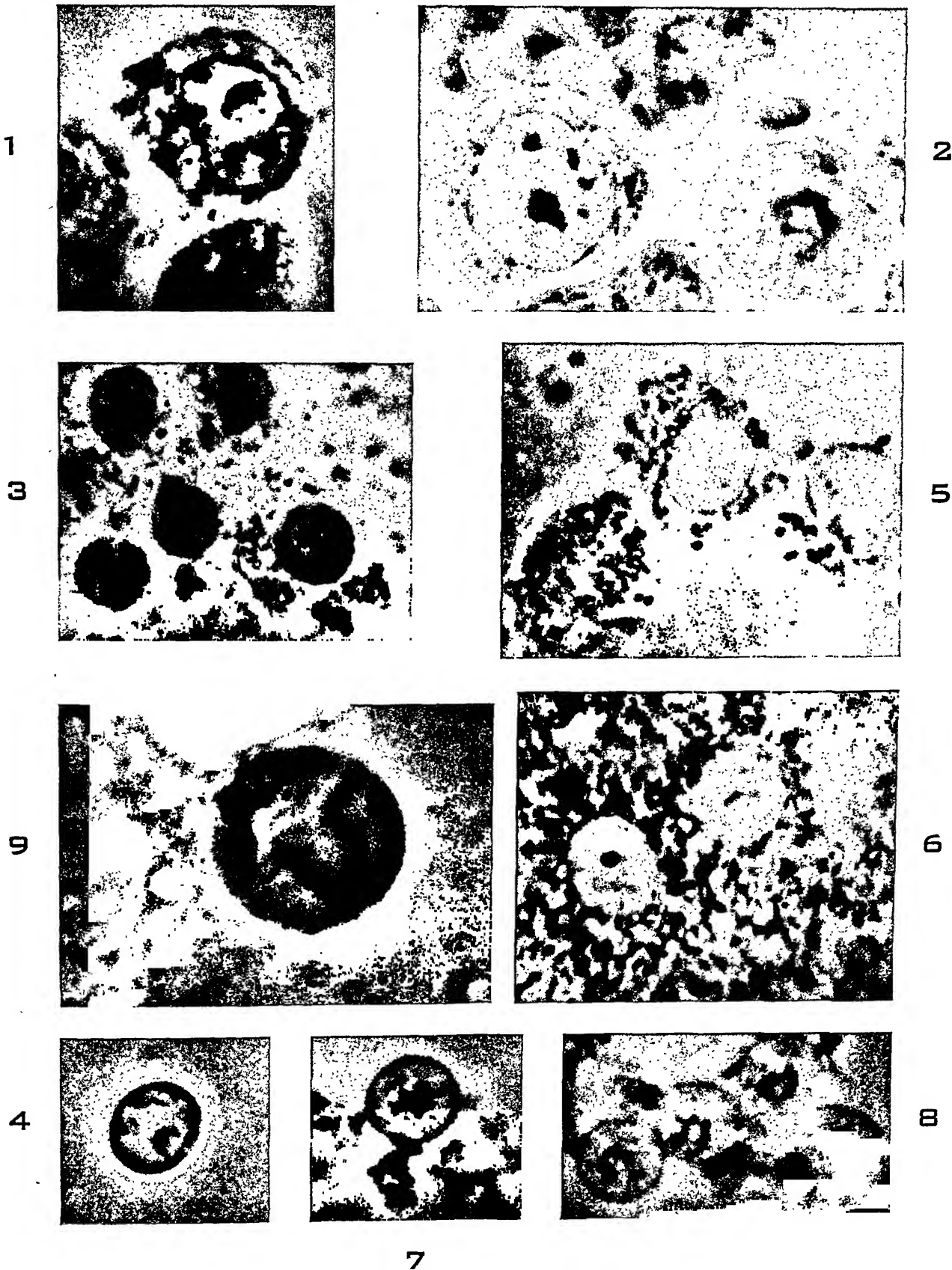
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DESCRIPTION OF PLATES

PLATE 129

- FIG. 1. V2 carcinoma cell. The large nucleolus, the somewhat smaller karyosomes, the chromatin network, and the regular nuclear membrane are readily seen. PM. $\times 1400$.
- FIG. 2. Brown-Pearce carcinoma cells. The nuclear elements in the cell on the left show a picture similar to that in Figure 1; the cell on the right is in the mitotic phase. The chromosomes (slightly out of focus) are surrounded by a bright halo. In the protoplasm of the mitotic cell there are numerous very small granules. Above the two tumor cells, several erythrocytes are seen. PM. $\times 1400$.
- FIG. 3. Kidney cells of the frog in distilled water. The nuclei are hazy and homogeneous; the nucleoli are clearly visible. PM. $\times 1400$.
- FIG. 4. Naked nucleus in a suspension of a breast carcinoma of a mouse. The nuclear membrane is double-contoured and brilliant; there is a bright diffraction ring around the nucleus. PM. $\times 1400$.
- FIG. 5. Kidney cells of the frog in molar NaCl. The mitochondria and the storage granules are relatively small; the nuclei are swollen and hazy; and the nucleoli have disappeared. PM. $\times 1400$.
- FIG. 6. The same cells as shown in Figure 5, just after replacement of the molar NaCl by physiologic saline solution. The nucleoplasm is still hazy; the nucleolus of one cell has reappeared. The storage granules and the mitochondria are slightly enlarged. PM. $\times 1400$.
- FIG. 7. Granulosa-cell tumor cell, showing a later stage of nuclear change after replacement of the molar NaCl by physiologic saline solution (for comparison with Figure 6). The rest of the chromatin reappears in the form of a few plump, black dots in the center of the nucleus. PM. $\times 1400$.
- FIG. 8. The same field as seen in Figures 5 and 6. The reappearing chromatin dots are arranged in ring shape. PM. $\times 1400$.
- FIG. 9. The same cell as shown in Figures 22 and 23 of the first study,¹ 2 minutes after ammonia was replaced by physiologic saline solution. The nuclear membrane is again visible, whereas the other elements did not change. PM. $\times 1400$.



Zollinger

Phase Microscopy, Induced Nuclear Alterations

PLATE 130

- FIG. 10. A Brown-Pearce carcinoma cell before (a), and 20 seconds after (b), replacement of physiologic saline solution of pH 7.0 by an isotonic saline solution of pH 10.2. The same changes are present as were produced by ammonia: enlargement and haziness of the nucleus, and swelling of the mitochondria. PM. $\times 1400$.
- FIG. 11. Kidney cells of the frog in 10 per cent formalin. The nuclear membranes, the nucleoli, and the chromatin networks are double-contoured and brilliant. The cellular membranes are out of focus. PM. $\times 1400$.
- FIG. 12. Brown-Pearce carcinoma cells after replacement of distilled water by 10 per cent formalin. The protoplasm has already shrunk, and the nucleolus and the chromatin network are beginning to reappear. PM. $\times 1400$.
- FIG. 13. Brown-Pearce carcinoma cells after molar NaCl has been replaced by 10 per cent formalin, which has just reached the cells. The chromatin reappears, and the granules have shrunk. PM. $\times 1400$.
- FIG. 14. A cell of the same suspension as shown in Figure 13, 1 minute later. The nucleus, the chromatin, and the nucleolus are brilliant; the chromatin network is very loose. There is a marked shrinkage of the protoplasm. PM. $\times 1400$.
- FIG. 15. Kidney cells of the frog in 5 per cent acetic acid. The mitochondria are enlarged and the storage granules have disappeared. The nucleus is brilliant, and the cellular membrane is no longer visible. PM. $\times 1400$.
- FIG. 16. A rabbit sarcoma cell in 3 per cent potassium bichromate. Strange, irregular, black chromatin dots are scattered throughout the nucleoplasm (intermediate stage). PM. $\times 1400$.

10A



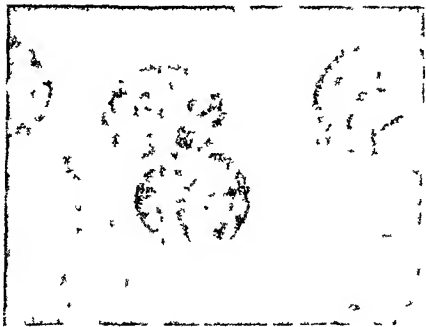
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12



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16



15



PLATE 131

FIG. 17. Brown-Pearce carcinoma cell. Intermediate stage, caused by 3 per cent potassium bichromate. PM. $\times 1400$.

FIG. 18. Brown-Pearce carcinoma cell with mitotic figure. The cell is enlarged; around the chromosomes there is a bright space. The cytoplasm contains numerous small, black granules and a few storage granules. PM. $\times 1400$.

FIG. 19. Lymphosarcoma of the mouse. These cells are not very useful for PM studies because they are too spherical and their bright diffraction ring prevents a distinct observation. The cell in the middle is in the telophase. PM. $\times 1400$.

FIG. 20. Mitotic Brown-Pearce carcinoma cell after substitution of the physiologic medium for distilled water. There is visible a distinctly outlined central vesicle similar to the hazy type of nucleus in distilled water. The protoplasmic granules are enlarged. PM. $\times 1400$.

FIG. 21. Mitotic Brown-Pearce carcinoma cell in molar NaCl, presenting the same picture as shown in Figure 20. PM. $\times 1400$.

FIG. 22. The same cells as shown in Figure 21, after replacement of the molar NaCl by physiologic saline solution. The chromosomes have reappeared, but they are rather plump. The perichromosomal halo is larger and more distinct than in normal cells. PM. $\times 1400$.

FIG. 23. Thin, curled fibrils of chromosin, extracted from cells under the coverslip by molar NaCl and precipitated by physiologic saline solution. PM. $\times 1400$.

17



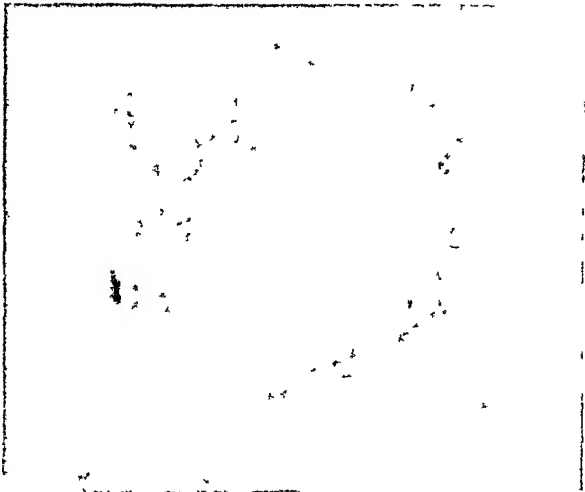
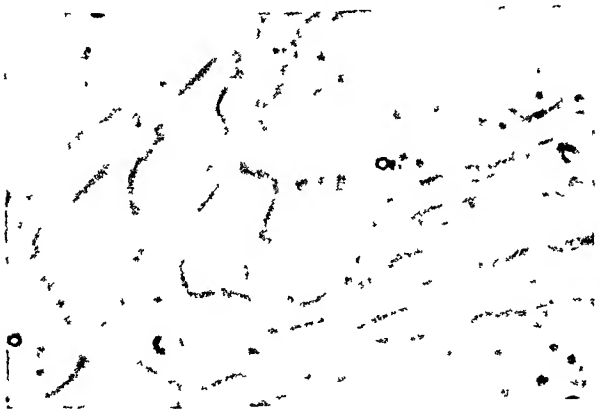
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EXPERIMENTAL ARGYROSIS

IV. MORPHOLOGIC CHANGES IN THE EXPERIMENTAL ANIMAL *

CHARLES T. OLCOTT, M.D.

(From the Department of Pathology of Cornell University Medical College and the New York Hospital, New York, N.Y.)

The term argyrosis is used to include all of the changes produced by the deposition of silver in tissues rather than argyria, as the latter is often limited by usage to pigmentation due to this metal.

In 1927 it was stated by Gettler, Rhoads, and Weiss¹ that the lesions of human argyrosis had never been even approximated in any experimental animal. The following experiments are presented to fill this void. The present communication reports the morphologic study of other organs that has already been begun for the eye.²

Microscopic slides from the "blue man," a case of generalized deposition of silver with autopsy report by Gettler, Rhoads, and Weiss¹ (1927), have been studied with care. In this case, as well as in the other 19 cases reported with reasonable completeness, the lesions of the kidney are among the most advanced changes found in any organ.

PREVIOUS EXPERIMENTS

Previous experiments in the production of argyrosis have been limited by several factors. The first of these is the method used for introduction of the silver. I have repeated the experiments of others such as Stewart and Parker³ (1926), who found that the results following the injection of colloidal silver were essentially similar to those following injection of any other colloidal substance. There is no evidence that this procedure is followed by generalized argyrosis. The most serious defect of previous attempts to study pigmentation by silver has been the short period of time during which the metal was given the experimental animals. Huet,⁴ in 1873, fed solid silver nitrate to a rat for 14 months and found that the eyes became dark, but his study of the tissues was inconclusive.

EXPERIMENTAL PROCEDURE

I have given silver solutions to guinea-pigs, mice, rabbits, and rats. The guinea-pigs did not live long enough to give positive results. This is in accord with the observations by Huet⁴ that this animal is un-

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satisfactory for this purpose. Mice and rabbits tolerated silver solutions for long periods and the lesions were in general similar to those found in rats, but the rat was chosen as the most convenient experimental animal for intensive study.

Solutions either of silver nitrate alone or of silver chloride with about three times as much sodium thiosulfate as silver salt have been used. Various concentrations of these salts were tried. When rats were given 1 per cent of the silver salts, they did not survive. Two rats were kept alive for over 500 days with no other fluid intake than 0.4 per cent solutions of silver chloride in sodium thiosulfate, but this dosage usually was found to be excessive. In early experiments, silver salts also were given in minute amounts but it was soon found that the life span of rats was not shortened by giving 1:1000 solutions of either of the silver salts without any other fluid and this strength was adopted as standard. The solutions were kept in dark bottles from which the rats could drink as much as desired.

Silver solutions were given to about 2 of every 3 animals, usually starting shortly after weaning, the other animals being given water to serve as controls. Except in a few instances, the silver solutions were continued throughout life. In a few rats, silver was stopped and water was given subsequently. There was never a recognizable decrease in pigmentation following this procedure.

The rats were given dog chow, as much as desired, with either one of the silver solutions or water, and nothing else. This diet has proved adequate for life, a large increment in growth, and high reproductivity in both sexes. The animals were weighed at intervals of approximately 2 weeks. The average weights of rats on silver was about the same as for control rats on water. There is no evidence that death ever was caused or even hastened by the use of silver salts in concentrations of 1:1000 instead of water.

STUDY OF RATS DURING THEIR LIFE

The rats given silver solutions showed no characteristic pigmentation of their skin except that caused by accidental contamination. Their eyes became progressively darker, apparently due to deposition of metallic silver or silver salts in the choroid layer. This was the most apparent change during life and has been described elsewhere.² A consideration of the weight of the animals and other physiologic changes will be postponed until a later date.

AUTOPSY FINDINGS IN RATS INGESTING SILVER

Most of the rats were killed with ether but a few were examined after spontaneous death, which almost always was due to respiratory

disease. The following observations are based on a study of slides from over 300 rats.

Fixation

Material fixed in a 10 per cent solution of formaldehyde demonstrates silver moderately well. With Kaiserling I solution⁵ the silver is more clearly shown than with plain formaldehyde. Bouin's fluid has been found to be much the best fixative tried. Zenker's fluid cannot be used for staining silver as the necessary removal of the mercuric salt with iodine and sodium thiosulfate discharges silver along with it. The removal of silver and special staining technics will be referred to when discussing the histologic features of various organs.

Skin. The skin showed no changes on macroscopic examination except for the pigment due to accidental contact with solution from the water bottles. Microscopically, no pigment was found in the epidermis, but granules characteristic of silver or silver oxide might be found in small numbers in the corium, especially around the sweat and sebaceous glands.

Tongue. On gross examination the tongue usually was black, as were also the teeth. Pigment usually was present on the free surface of the horny layer of the tongue, but not in the epidermis itself. It was found in the tips of the papillae and along the blood vessels of the corium. It also was found between the muscle fibrils in fine strands which usually represented the walls of capillaries.

Salivary Glands. The salivary glands were characteristically very black on gross examination. Microscopically, silver might be deposited in large amounts. There usually was more silver around the ducts of the serous glands than around the acini of either the serous or mucous glands.

Thyroid. The thyroid was regularly gray to black on macroscopic study. Granules of silver might be found in such abundance on microscopic examination as to form an uninterrupted band of brown pigment in the walls of the capillaries adjacent to the base of the glandular cells. The pigmentation disappeared when the slides were treated with iodine and thiosulfate. The structure of the thyroid acini usually was unchanged (Fig. 1).

Parathyroid. The parathyroids were the site of deposition of moderate numbers of granules which usually were arranged along the small vessels in such a way as to separate small nests of cells from one another (Fig. 1).

Lungs. No changes due to ingestion of silver were found in the lungs on gross examination. Many microscopic sections have been examined but silver never has been satisfactorily demonstrated, perhaps, in part at least, because the presence of hematogenous and anthracotic

pigment granules renders the interpretation uncertain. Especially in old animals, pulmonary changes of all degrees may be present.⁶

Heart. The heart usually was gray at autopsy. Microscopically, granules of silver were deposited chiefly in the walls of small vessels. They varied greatly in number, but in rare sections might form moderately long strands adjacent to the muscle cells. These strands appeared to represent the walls of the capillaries. There was frequently a definite hypertrophy of the left ventricle of rats that had ingested much silver for long periods.

Blood Vessels. A few granules of silver sometimes were present in the adventitia of the aorta.

Lymph Nodes. Some cervical and axillary lymph nodes appeared black on macroscopic examination. Microscopically, these nodes contained very few pigment-containing histiocytes. The reticular network at the base of the cells lining the sinuses of the nodes might be the site of deposition of fine brown granules. In the nodes of the mediastinum and pericardium the same reticular pattern was seen, but they contained many more almost black histiocytes than the nodes previously described. In the intra-abdominal nodes, pigmentation of both types was still more advanced and extremely large masses of very large and very black histiocytes might be seen.

When lymph nodes from rats that received water were stained with Foot and Foot's stain,⁷ the capsules were dark. There was a little fine reticulum in the secondary follicles with much coarser fibrils in the sinuses, especially around the blood vessels. Similar, but less clearly defined results were obtained in tissues from animals given water when such tissues were stained with stronger solutions of silver nitrate and carbonate ("platafuerte") than required for the usual del Río-Hortega's method⁸ for microglia and oligodendroglia. Dr. N. C. Foot has examined some of my slides and believes that the fine granules of silver deposited on the reticular fibers in the nodes of rats ingesting silver present a different picture than that in silver-stained tissues from rats receiving water. In the former he postulates a physical adhesion of the granules to the fibers while in the latter he thinks the slides indicate a definite chemical combination.

Liver. The liver was slightly dark on gross examination but showed no other definite changes. Under the microscope, however, it showed changes practically as characteristic and advanced as those found in the kidneys. In fact, in rare animals there was pigment in the liver and none in the kidneys. Pigment was found in varying amounts in Kupffer cells, and, as the bulk of this pigment could be decolorized with iodine and thiosulfate, it was clear that most of it was silver. Histo-

cytes in the portal canals might be similarly pigmented. The earliest and most constant finding was the presence of brown pigmentation interspersed with black spots that involved the entire thickness of the portal veins. The hepatic veins also were stained with silver but to a lesser degree than the portal veins. The parenchyma was not significantly changed by giving silver. Advanced congestion was no more common in treated than in control animals (Fig. 2).

Slides from control rats receiving water which were stained with Foot and Foot's stain ⁷ showed a diffuse reticulum throughout the liver. These strands were long and narrow and bore no resemblance to Kupffer cells. The walls of the portal veins had the red color characteristic of collagen rather than the black color of reticulum. It would appear from this staining reaction and confirmation with Mallory's connective tissue stain that the deep pigmentation of the portal veins of the rats ingesting silver was due largely to deposition of silver around collagenous fibers. Wilder's stain ⁹ also showed an extensive reticular network. Slides from rats receiving only water stained for microglia showed a few Kupffer cells of characteristic structure but no staining of the reticulum.

Pancreas. The pancreas was one of the most deeply pigmented parts of the body. Whereas the mesentery except for the nodes was wholly unstained, the pancreas was gray to black. Microscopically, pigment that could be decolorized by iodine and thiosulfate was regularly found in the connective tissue around the medium-sized ducts, through the entire wall of the veins, and in the adventitia and between the muscle cells of the arteries. In no other organ were the walls of the arteries stained so clearly. There were varying amounts of pigment around the acini, but rarely any pigment in the acini themselves. The small arteries of the islands of Langerhans were often deeply pigmented. In one rat, coarse, fibrous, pigmented strands were found to traverse the pancreas. This was considered an accidental finding.

The urine of a moderate number of rats on silver was tested for sugar but always with negative results.

Gastrointestinal Tract. When the peritoneal cavity of a rat that had received much silver was explored, the cardiac end of the stomach had a normal color but the pyloric portion and all of the intestines might be very dark. When the stomach was opened, the mucosa of the pylorus usually was dark, but there was no discoloration at the cardiac end. Microscopically, there was rarely any pigment on the surface of the squamous epithelium of the esophagus or of the cardiac portion of the stomach, but there might be a very little pigment in the papillae under the epithelium. It was the rule for the free surface of the

glandular epithelium to be covered by a thin row of granules that could be decolorized by iodine and thiosulfate. This pigment usually surrounded the most superficial epithelial cells and there might be a very few fine granules, especially in the wall of the small blood vessels, between the deeper epithelial cells. In rare sections, there might be a solid row of dark granules between the mucosa and the submucosa and around the larger veins. The mucosa of the intestine was often moderately pigmented on gross examination. On microscopic examination of the small intestines there might be a continuous line of pigment directly under the superficial epithelium of the papillae. It was the rule to find large amounts of pigment within histiocytes in the papillae and smaller clumps of pigment in the submucosa. Many fine granules might be present in the walls of the smaller and larger blood vessels. In the cecum and in the rest of the large intestine there usually were many pigment-containing histiocytes in the tissue between the mucosal crypts. Pigment might be deposited in fine granules under the epithelium. It might be found in moderate to large amounts in all layers of the walls of the arteries and veins.

Spleen. No definite change was present in the spleen on macroscopic examination. Microscopic examination showed that silver often was deposited in moderate amounts as fine granules in the inner layer of the capsular tissue and in larger amounts in the trabeculae. No definite perivascular arrangement could be established. These granules were decolorized with iodine and thiosulfate and clearly were silver. In parts of the spleens of some rats a fine network composed of granules surrounded each cell. This network was especially evident in the outer zone of the malpighian bodies and the pigment could be removed with iodine and thiosulfate (Fig. 3).

Sections from a rat receiving water, when stained with Wilder's technic,⁹ showed similar staining of the reticulum of the pulp and of the trabeculae. Sections from a rat receiving water, when stained with Foot and Foot's technic,⁷ showed the same reticulum but it was somewhat less clearly defined. It was similar to that found in the spleen of man.¹⁰ Similar but even less clearly defined staining of the reticulum may be obtained with the technic for microglia and oligodendroglia ("platafuerte"). Pigment of an entirely different type was found in the phagocytic cells of the splenic pulp of animals receiving water as well as of those receiving silver. This was not decolorized with iodine and thiosulfate but was stained by Pearl's reaction for iron, and it was clearly of hematogenous origin. In these respects it was in contrast to the pigment of the Kupffer cells of the liver.

Adrenal Glands. The adrenal glands were slightly darker in animals receiving much silver than in the controls, but were never as black as the thyroid and pancreas. On microscopic examination, there might be moderate numbers of granules in the zone that included the inner part of the capsules and the peripheral part of the cortex, with only a very few granules in other parts of the cortex. The groups of cells in the medulla were surrounded regularly by brown strands often containing a practically continuous line of granules. The granules were found chiefly, perhaps exclusively, in the walls of the blood vessels, where they were characteristically deposited just under the endothelium. They could be decolorized with iodine and thiosulfate. When tissues from the adrenals of rats receiving water were stained with Mallory's or Masson's technics, the characteristic tinctorial reactions for collagen were seen most clearly in the capsules and in the tissue between the groups of cells of the medulla. Collagen thus was demonstrated in the same locations as was the greatest amount of silver in the spleens of rats ingesting silver (Fig. 4).

Testis. The testes of animals receiving silver were unchanged. Even when the rats had been given large amounts of the metal, none was demonstrated in the testis, but there might be very few fine granules in the seminal vesicles. The ingestion of silver did not modify the appearance of the spermatozoa, and male rats receiving silver showed no diminution of fertility.

Uterus and Ovary. In the uterus, very small amounts of silver in characteristic granular form might be found in the walls of the arteries and in the stroma. Almost all of the pigment in the stroma of the ovary was of hematogenous origin and stained for iron. Female rats receiving silver showed no definite loss of fertility.

Bone Marrow. In an occasional section of the bone marrow there were a few black granules. These might represent silver, but the possibility that even these granules represented hematogenous pigment could not be ruled out. The marrow of animals on silver and on water appeared the same.

Joints. Granules characteristic of silver were not found in the cartilage or the synovial membrane.

Striated Muscle. There were a few fine granules between the strands of striated muscle. This pigmentation was minimal and was clearly defined only in the walls of the fine capillaries between the strands of muscle.

Brain. Granules of silver never were recognized in the brain tissue or in the vessels of the brain itself. There was regularly, however,

an abundant deposition of granules in the choroid plexus, which might form an almost unbroken line between the endothelium of the blood vessel and the overlying ependyma. This pigment was rendered colorless by iodine and sodium thiosulfate. The ependymal cells were never stained. Rarely, there were a few granules in the intima of the veins of Galen. Small vessels in the pineal gland contained granules resembling silver in all respects. When the choroid plexus of a rat receiving only water was stained by Foot and Foot's technic,⁷ there was a solid black line in the walls of the fine blood vessels.

Pituitary Body. In rats receiving much silver, the fine blood vessels of the pars nervosa of the pituitary body were regularly yellow to brown with darker granules in their walls, especially just under the endothelium. The walls of the blood vessels of the pars distalis were much less frequently pigmented; when they were, they contained fewer granules than did the pars nervosa.

Bladder. The bladder showed no pigment in the epithelium, but just beneath the epithelium there might be a continuous line of pigment granules. In the fibromuscular layer, separate fine granules often were found along fibrillary structures with a few clumps of granules in cells, some of which were histiocytes.

Kidney. On gross examination of rats that had received silver, the kidneys often were very dark. It was common to find still darker spots which represented the glomeruli when the animal had received much silver. The epithelium of the pelvis was not stained. In the experimental animal, as in man, the kidney was usually the most advanced site of deposition of silver pigment. Silver was deposited in the basement membrane of the glomerular tuft, which might be pigmented to any degree from light yellow to almost solid black. The membrane appeared more sharply delimited than with any method of staining that I have seen. Lesser amounts of pigment were found in the basement membrane of the collecting tubules or in the wall of the small blood vessels between the tubules. There might be, in rats receiving much silver, a complete line of pigment surrounding the tubules. The deposition of silver adjacent to the basement membrane of the convoluted tubules in the rat was usually more spotty, the membrane of some tubules being uniformly pigmented, while in other tubules it remained practically unstained. Sometimes, more pigment was present around the distal than around the proximal convoluted tubules. In other slides, there appeared to be more staining of the membranes of the inner third of the cortex (the subcortical zone) than in the outer two-thirds of the cortex. In rabbits, the deposition was similar to that in rats. In mice, it was similar except

that there tended to be more pigmentation of the basement membranes of the convoluted than of the collecting tubules.

The density of pigmentation of the glomerular basement membrane was, in general, directly related to the duration of ingestion of the silver salts, and to the amount of salt ingested, but there was no close correlation (Figs. 5 and 6).

Special stains were made on sections of the kidneys of rats receiving water only and also of rats receiving silver solutions. In the latter group, staining was done on tissues with and without previous bleaching with iodine and sodium thiosulfate. Mallory's connective tissue stain, preferably with tissue fixed in Zenker's solution and acetic acid, showed that the two areas where silver was deposited in greatest amount in the kidneys of rats ingesting large amounts of silver appeared to be made up of collagen closely associated with, if not actually forming, vascular walls. In 2 rats that had ingested large amounts of silver, the glomerular basement membrane was heavily pigmented with silver. After this pigment was removed with iodine and thiosulfate, the tissue was stained by Mallory's technic and the basement membrane was found to be definitely thicker than in the kidneys of rats that had received only water.

The glomerular membrane of rats given only water to drink was not stained continuously with Foot's modification of Bielschowsky's method,¹¹ or by Foot and Foot's method 3B,⁷ although with the latter technic there might be a very few fine strands between the glomerular tufts. With del Río-Hortega's lithium carbonate method¹² the basement membrane was not stained, but it was stained by Wilder's method.⁹ All of the silver methods cited demonstrated a network of reticulum fibers, apparently around the fine vessels between the tubules, which was more fibrillar than the smooth basement membrane found in the otherwise unstained tissues of animals ingesting silver for long periods of time.

The decolorization or removal of silver from the organs of the experimental animal by various agents is a matter of some interest. Gram's iodine alone (I used iodine, 1; potassium iodide, 2; water to 100) caused only a small amount of bleaching of the deposited silver. Sodium thiosulfate alone had no effect. Gram's iodine followed by sodium thiosulfate regularly bleached the silver. The residuum is colorless but has some doubly refractive qualities. The color can be made to return by the use of a photographic developer. In other words, the above treatment seems to form a latent or "leuko-product" rather than to dissolve the silver. Following the use of potassium cyanide, the developer does not cause return of pigmentation and it is postulated that in this case the process is true solution. Treat-

ment with nitric, hydrochloric, or sulfuric acids, ammonium hydroxide, sodium carbonate, mercuric chloride, or potassium ferri-cyanide caused no change of appearance of the silver. Most of the above tests have been made on tissues fixed with Bouin's fluid, some also on tissues fixed with formalin. The above chemicals were used in about 5 per cent concentration for several hours.

An attempt to remove silver by a method described by Mallory and Wright¹³ has been unsuccessful. They did not indicate the strength of solution to be used. When slides from one of my rats were kept in a mixture of equal parts of 1 per cent potassium ferri-cyanide and 1 per cent sodium hyposulfite for 36 hours, there was no apparent bleaching of the pigment. Our unsuccessful attempt to remove silver from the tissues of living rats has been reported elsewhere.¹⁴

In a number of rats, largely males, that received silver there have been from small to large amounts of albuminous material in the convoluted and collecting tubules. This condition was found also in animals given only water and appeared to be correlated more closely with the age of the rat than with whether they had received silver. The maximum amount of dilatation of tubules by albuminous material has been found in "reduced" kidneys¹⁵ (operations by Dr. William Dock) of animals receiving either silver or water; that is, in animals in which one kidney was removed and parts of the other injured with heavy clamps.

DISCUSSION

Entrance of Silver into the Body

A study of microscopic sections gave no evidence of absorption of silver through the gastric mucosa. On the other hand, the large amount of silver found in the villi of the small intestine indicated that the intestines might be the point of entrance of silver into the organism. The possibility was strengthened by the fact that the silver was found deeper in the wall of the large than in the small intestine. As previously noted, there was very much more silver in the mesenteric and other intra-abdominal lymph nodes than in the cervical or axillary nodes of the rat. There was no evidence to indicate whether silver enters the circulation of the rat principally through the portal vein or through the lymphatics, but it is probable that it enters through both channels.

Removal of Silver from the Body

The presence of silver in the basement membrane of the glomeruli and tubules and under the epithelium of the bladder gave only a faint suggestion of possible excretion of silver through the urinary

tract. I did not find recognizable amounts of silver in the urines of a number of rats which I tested for silver. In human cases of argyria, silver has been found by spectroscopic analysis of the urine by Blumberg and Carey,¹⁶ but was not found in the urine by ordinary chemical analysis by Aub and Fairhall,¹⁷ or in the large bladder stone reported by Klinck¹⁸ and later referred to by Jacobsen¹⁹

Nature of Silver in the Rats

The nature of the fine black granules described in the various organs is uncertain but there appears to be no way in which they are incompatible with either metallic silver or silver oxide.

Site of Deposition of Silver

There are certain locations in which silver is regularly found in the experimental animal:

(1) In histiocytes of lymph nodes and liver. As previously noted, the phagocytes of the lymph nodes may be uniformly black. This pigmentation is most advanced in the abdominal nodes, next most advanced in the mediastinal and pericardial nodes, and much less evident in the cervical and axillary nodes. It is present in the Kupfer cells and in the phagocytes of the portal canals of the liver, but apparently absent in the histiocytes of the spleen. The histiocytes which contain pigment are characteristically much blacker than the stained reticulum fibers. In fact, the two types of cells are moderately sharply differentiated by their reaction to ingested silver.

(2) In association with the reticulum fibrils of the sinuses of the lymph nodes and the periphery of the malpighian bodies of the spleen. In pigmentation of this type, the reticulum shows tinctorial reactions which are in general very close to those found when silver is used as a stain on sections from control animals receiving only water. The results have not indicated any predilection for staining of elastic fibrils in the rats ingesting silver, as is reported by many authors in human material.

(3) In close approximation to the blood vessels. This deposition is especially evident in the tissue between the endothelium and epithelium of the thyroid, choroid of the brain, and the glomeruli and tubules of the kidney. It is also found near or in fine blood vessels in such different organs as the pancreas, adrenal medulla, pars nervosa of the pituitary body, choroid of the eye, and in striated muscles.

The staining of the basement membrane of the renal tubules and the fine vessels between the tubules is more uniform in animals ingesting silver than in the tissue of animals receiving water after they have been stained with Foot and Foot's stain. In fact, with the latter

the tissue between the tubules resembles a network of reticulum fibrils more than it does a homogeneous membrane.

The most striking variation in staining is found in the glomeruli. Animals ingesting much silver show an advanced uniform staining of the glomerular basement membrane, whereas the structure is only rarely stained with various silver technics. Very few authors have stained the basement membrane of the glomerulus with any silver method. Clara²⁰ found that the membrane was stained by Pap's but not by either Bielschowsky-Maresch's or del Río-Hortega's technics. Wilbur²¹ found that the glomerular membrane was not impregnated by Orlandi's silver technic. Bensley and Bensley²² stated that the basement membrane of the glomerulus was not stained with silver. As previously noted, Wilder's technic does give a good staining reaction with the basement membrane of the glomerulus.

Conditions are almost exactly reversed with respect to the peripheral part of Bowman's capsule. In the rats ingesting silver this structure was not stained. Wilbur,²¹ using Orlandi's silver technic, found no impregnation in the thin inner layer of Bowman's capsule, but did report impregnation in its thick outer layer. Most of the usual silver technics readily stain at least the major part of Bowman's capsule.

Silver pigmentation has never been demonstrated in any epithelium of the rat. This is in accordance with the findings in man.

Comparison of Lesions Due to Silver in the Rat and in Man

Unless otherwise noted, the human lesions to be referred to are from the description by Gettler, Rhoads, and Weiss,¹ 1927.

In the rat and man the epithelium of the skin is unpigmented, while pigment is deposited in both species around the sweat and sebaceous glands.²³ Silver has been described in the human thyroid by Jahn²⁴ and Dohi,²⁵ among others. I have found no reference to the deposition of silver in human parathyroids. Silver was demonstrated by Gettler, Rhoads, and Weiss along connective tissue fibrils in the lungs and under the pleural mesothelium, but I have never been sure of it in the rat. The slate-gray color and microscopic picture in the heart are essentially the same in the rat and in man. I found no silver in the intima or media of the rat's aorta although it has been found in man. The findings in the rat's liver are essentially the same as in man except that there is undoubted silver in the Kupffer cells of the rat, while it is absent from these cells in the cases described by Gettler, Rhoads, and Weiss,¹ and by Klinck.²⁶ In both species intense pigmentation of the pancreas is recognizable on gross and micro-

scopic examination, and the same pigmentation is found also in the gastrointestinal tract. In man and in the rat, silver is often deposited in the spleen and in various lymph nodes. Tobler²⁷ described silver in the human adrenal, but I have found no reference to the selective localization in the medulla that is shown so clearly in rats. Silver is present in the striated muscles of the rat as well as in man. The most notable difference between the tissues in man and the rat is the complete lack of deposition of silver in the testis of the latter. The deposition in the choroid plexus of the brain in both species is practically identical. My rats have never shown any recognizable silver in the walls of the vessels of the brain and meninges, whereas much pigment has been described in this location in man. In the rat, as in man, silver is present in the submucosa of the bladder. The most advanced deposition of silver in the internal organs of the two species is that found in the kidneys, especially in the basement membranes of the glomeruli.

SUMMARY AND COMMENT

When rats are given a 1:1000 solution of silver nitrate or 1:1000 silver chloride dissolved in about 1:300 sodium thiosulfate for long periods, there is intense pigmentation of many of the tissues. This pigmentation is most advanced in the basement membrane of the glomeruli, the walls of the vessels between the tubules of the kidney, the portal vein and other parts of the liver, the choroid plexus of the brain, and the choroid layer of the eye, and in the thyroid gland, but it is found in most of the body tissues.

The ingestion of silver salts in this concentration is unaccompanied by any lethal effect in the experimental animals, but a definite hypertrophy of the left ventricle frequently has been found in rats that had received much silver for long periods. Data with respect to this finding will be presented later.

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[*Illustrations follow*]

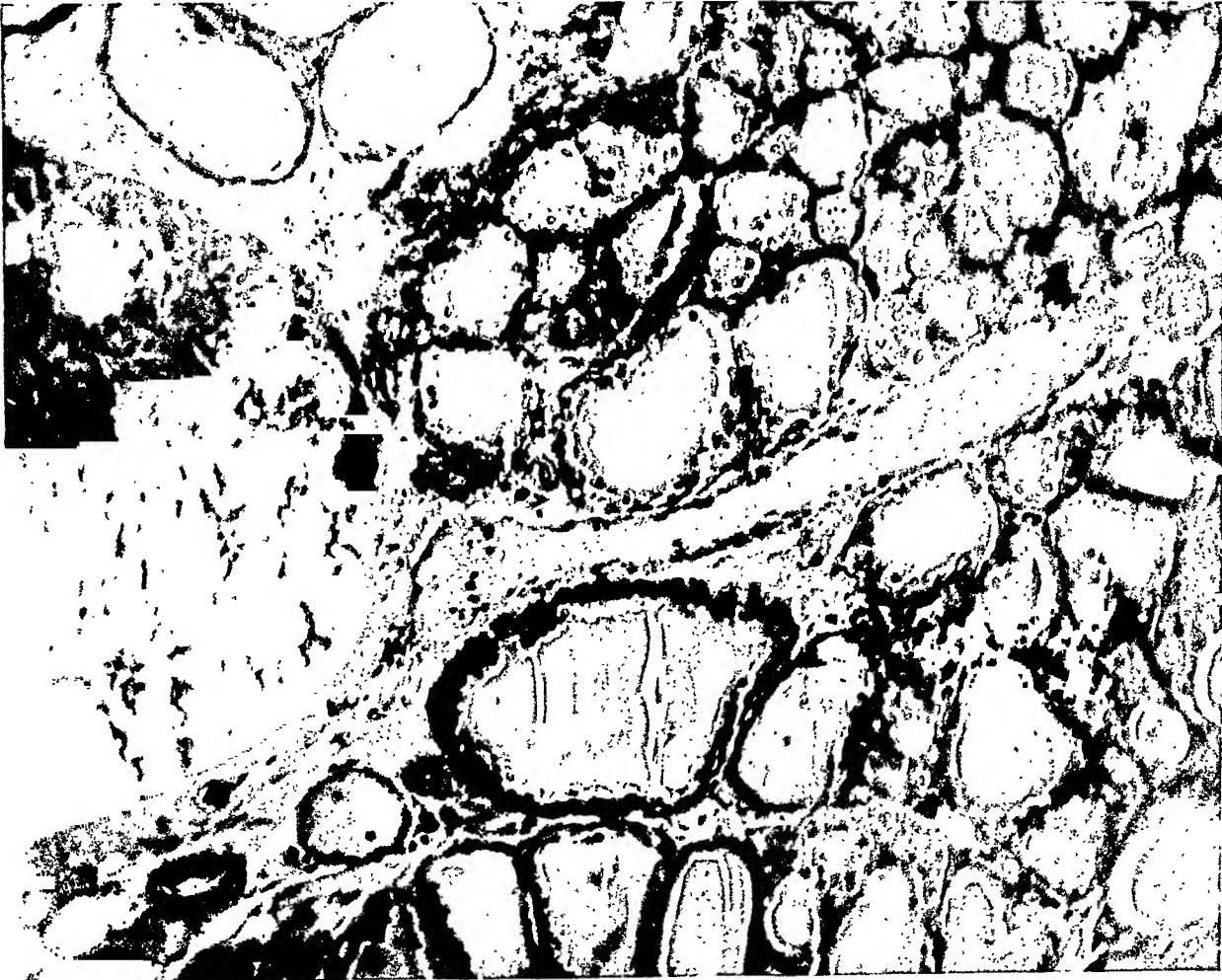
DESCRIPTION OF PLATES

PLATE 132

FIG. 1. Thyroid and parathyroid from a female rat, 22 months old, that received 13.3 gm. of silver nitrate in 615 days. The black pigment around the thyroid epithelium and in the walls of the small vessels of the parathyroid is silver. The pigmentation in the thyroid was rated as 3 plus. Hematoxylin and eosin stain. $\times 150$.

FIG. 2. Portal canal in the liver of a female rat, 26 months old, that received 17.2 gm. of silver nitrate in 757 days. The large structure below the center of the field is a portal vein with silver pigment throughout the entire thickness of its wall. Above this is seen the reticular network around two bile ducts and two large histiocytes with ingested silver pigment. A hepatic artery is shown in the upper left corner. The black spots in the structure of the liver lobules are almost all Kupffer cells. The pigmentation of the portal vein was rated as 3 plus. The tissue is unstained except for the picric acid of the Bouin's fluid used in the fixation. $\times 300$.

1



2

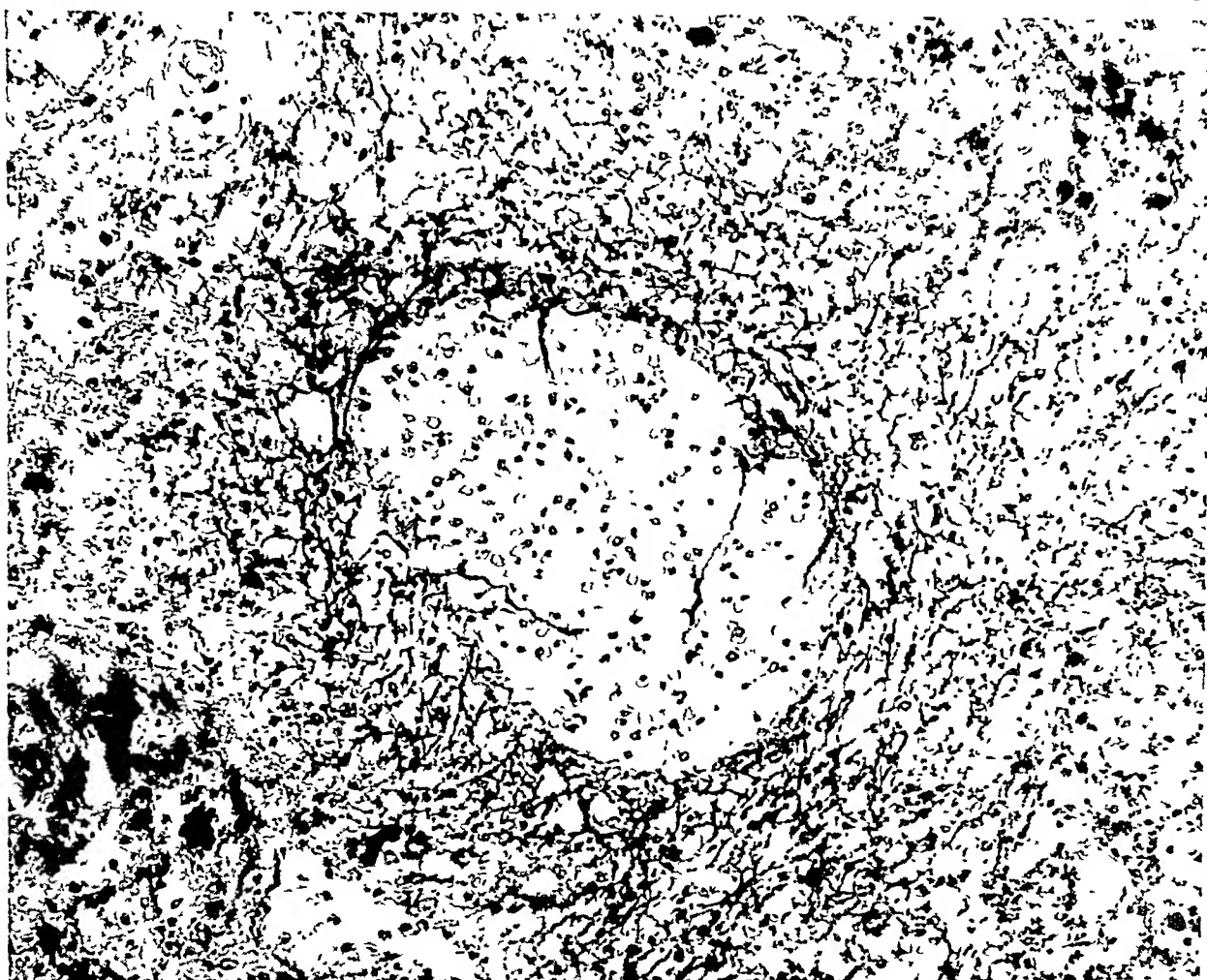


PLATE 133

FIG. 3. Spleen from the same rat as used for Figure 2. This section was unstained except by the ingested silver. The clear structure in the center of the field is a malpighian body, and the surrounding argyrophilic reticulum is clearly shown. Most of the pigment in the splenic pulp is of hematogenous origin. The splenic pigmentation was rated as 2 plus. \times 300.

FIG. 4. Unstained section of the adrenal of the rat from which Figures 2 and 3 were made. The pigmentation in the capsule and the reticulum around the groups of cells in the medulla can be seen readily, with only scant pigment in the cortex. The pigmentation of the adrenal medulla was rated as 3 plus. \times 55.

3



4

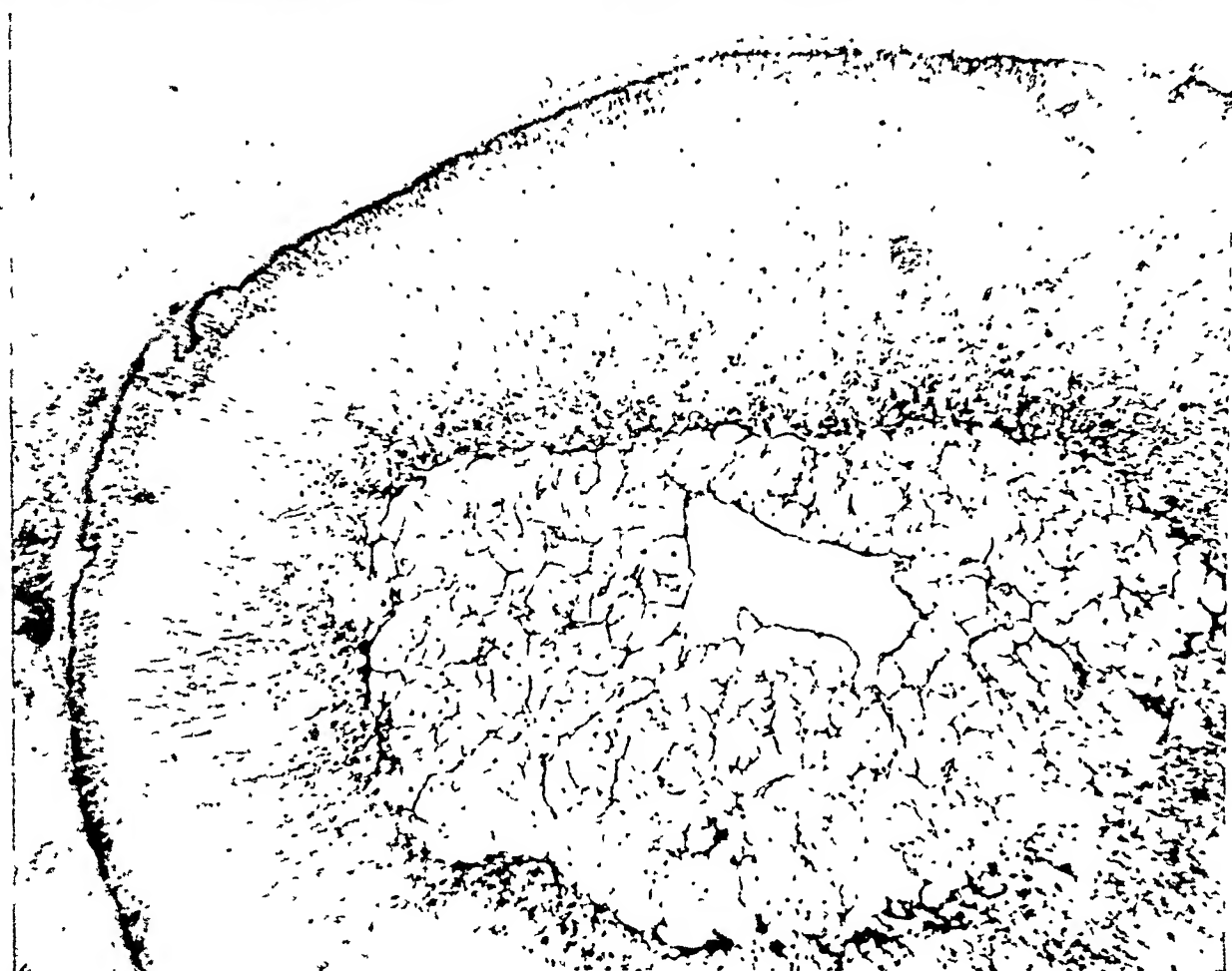


PLATE 134

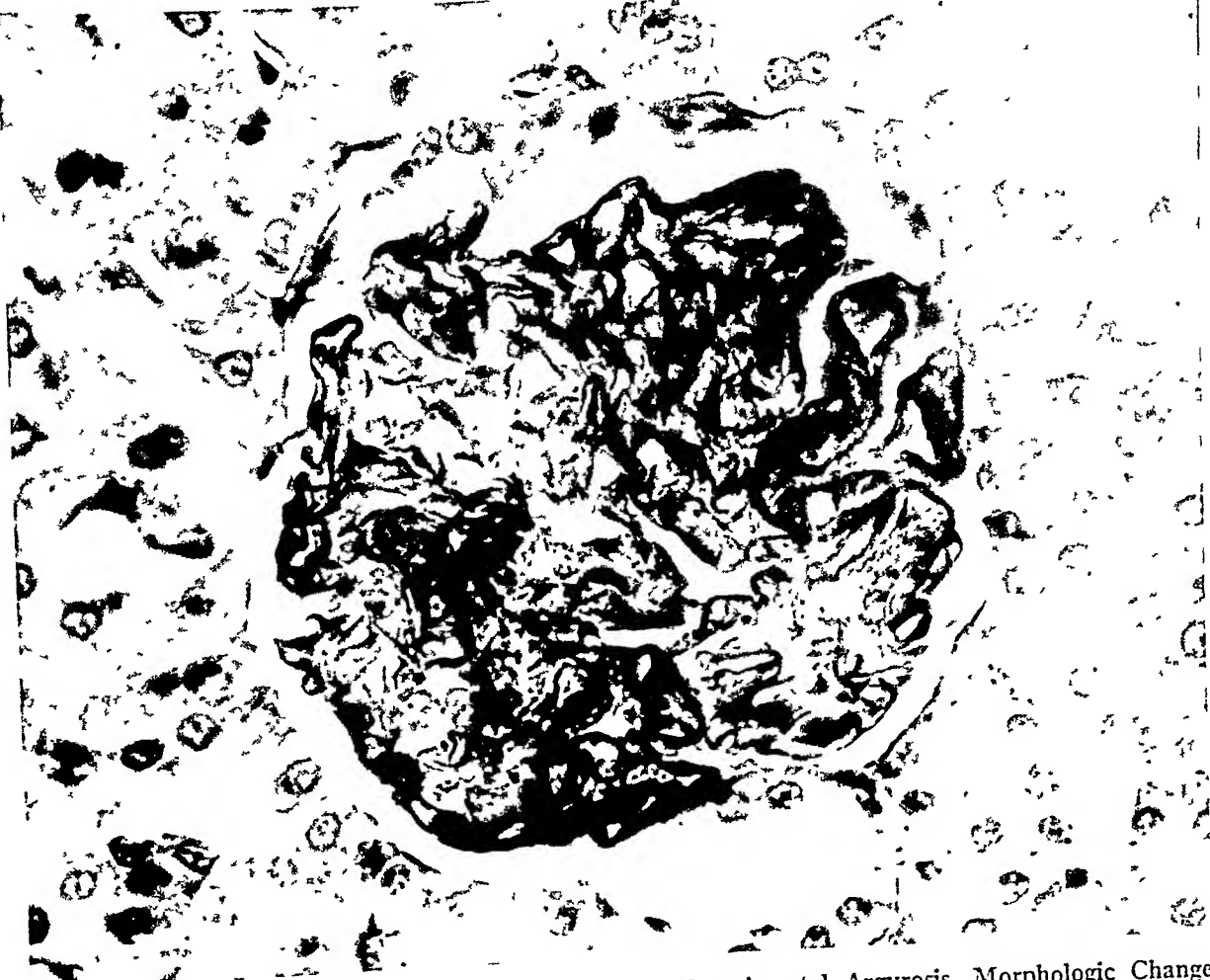
FIG. 5. Renal glomerulus of a male rat, 29 months old, that received 22.5 gm. of silver chloride in three times as much sodium thiosulfate for 733 days. This glomerulus was selected for photography because the presence of more than the usual number of blood cells in the glomerular capillaries made the glomerular basement membrane more evident than if the capillaries had been empty. In most kidneys from rats with as much silver deposition as this one (4 plus), the glomeruli are almost solid black. Hematoxylin and eosin stain. $\times 600$.

FIG. 6. Renal glomerulus from a male rat, 18 months old, that received 12.9 gm. of silver nitrate in 511 days. The pigmentation due to silver is advanced (3 plus) in the glomerular basement membrane, but there is none in the parietal layer of Bowman's capsule. Hematoxylin and eosin stain. $\times 600$.

5



6



Experimental Argyrosis, Morphologic Changes

OBSERVATIONS IN GUINEA-PIGS FOLLOWING INJECTION OF
SPECIFIC HEMATOPOIETIC SUBSTANCES DERIVED
FROM BEEF LIVER *

LEO M. MEYER, M.D., and ARTHUR SAWITSKY, M.D.†

*(From the Department of Therapeutics, New York University,
College of Medicine, New York, N.Y.)*

In 1906 Ziegler¹ studied the effect of x-radiation on a variety of animals and concluded, as quoted by Piney: "Myeloid leukemia is, therefore, the expression of peculiar hyperplastic processes in myeloid tissue, arising on account of a disturbance of the normal relationship between the lymphatic and the myeloid apparatus. This leads to myeloid metaplasia of the spleen and to flooding of the blood with myeloid cells." In 1933 Hubble,² in England, reviewed the hematologic findings associated with various endocrine disorders and reiterated Naegeli's theory that hematologic disorders were directly related to endocrine dysfunction. The work of Murphy and Sturm,^{3,4} Dougherty and White,⁵ Gardner and Dougherty,⁶ Furth and his co-workers,^{7,8} and others lends support to the rôle of the hormones in the production of leukemia in the experimental animal. In 1936 Wiseman, Doan, and Erf⁹ pointed out an apparent physiologic reciprocal relationship of myeloid and lymphoid tissue, *i.e.*, hyperplasia of one system resulted in hypoplasia of the other. In a series of experiments¹⁰⁻¹² with rabbits these authors demonstrated that nucleic acid derivatives stimulated myelopoiesis with myeloid metaplasia of the spleen and kidney, hyperplasia of the bone marrow, and an increased delivery of granulocytic cells into the peripheral blood. With this increased myeloid stimulation, lymphopoiesis was reduced. In another series of experiments they demonstrated that native proteins, *i.e.*, egg albumen and horse serum, stimulated lymphopoiesis and hyperplasia of lymphoid tissue in the lymph nodes and spleen. The bone marrow became hypoplastic. Large numbers of eosinophils and plasma cells were noted in the lymph nodes and bone marrow. An absolute lymphocytosis and a concomitant fall in myeloid elements were noted in the peripheral blood.

Cooke,¹³ in 1938, reported 11 cases of acute leukemia treated with beef bone-marrow extract. Four patients showed temporary clinical

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† Lederle Fellow in Hematology.

and hematologic remission, 6 showed clinical and hematologic arrest of a previously progressive course, and in 2, lymph nodes and spleen decreased in size. The bone-marrow extract was given on the hypothesis that acute leukemia is an acquired defect in normal marrow function characterized by the inactivation or destruction of one of the normal factors in hematopoiesis. Cooke suggested that the proliferation of immature marrow elements may be secondary to a maturation defect or to exhaustion of a normal inhibiting factor or mechanism.

In 1939 Miller, Wearn, and Heinle^{14,15} reported myeloid metaplasia and reticulo-endothelial stimulation in organs of guinea-pigs injected with an extract made from the urine of patients with myeloid leukemia. Following this, Miller and Turner¹⁶⁻²² published numerous reports of urinary extracts made from both myeloid and lymphoid leukemic patients, elaborating on the type reactions observed and the physical and chemical properties of the factors involved. The lymphoid stimulating substance, a carbinol, was called "lymphokentric acid" and the myeloid stimulating factor, a noncarbinol, "myelokentric acid." The two factors were claimed to be oxidation and reduction compounds of each other. The lesions produced in the "myeloid" animals were myeloid metaplasia of the spleen, myelopoiesis of the bone marrow, and proliferation of immature myeloid elements in the liver and adrenals. The lungs, kidneys, and lymph nodes were less frequently affected. The "lymphoid" animals exhibited hyperplasia of the lymphoid elements in the nodes and spleen, and proliferation of lymphocytes in the liver, kidney, and lungs. The adrenal medulla was involved less frequently. Bone-marrow effect was not mentioned.

In 1942 Heinle, Wearn, Weir, and Rose²³ reported the production of myeloid hyperplasia and metaplasia induced in guinea-pigs by extracts of urine from patients with myelogenous leukemia. They were unable to detect any difference between results obtained by the injection of extracts from patients with lymphoid leukemia and those of normal subjects. Heinle and his group^{23,24} believed that the myeloid factor is probably a protein or glycoprotein.

In 1943 Turner and Miller²⁰ reported the presence of similar factors extracted from normal beef liver. Erf, Turner, and Miller,²⁵ in 1946, reported the production of "myeloid reactions," "lymphoid reactions," "Hodgkin's reactions," and "slight lymphoid or monocytoïd reactions" from lipid extracts of human liver, spleen, and lymph nodes from cases of myeloid leukemia, lymphoid leukemia, Hodgkin's disease, and "normal" organs, respectively. Erf,²⁶ in 1947, injected lipid extract of "myeloid leukemic placenta" into guinea-

pigs and noted myeloid cell infiltrations in the liver, kidneys, and spleen. The bone marrow was hyperplastic. Normal placental extract produced no effect.

Miller and Turner^{16,17} hypothesized two related substances in the blood, mutually reciprocal in action. The myeloid substance stimulates myelopoiesis without maturation, and inhibits lymphoid proliferation, allowing lymphocytic maturation. Myeloid maturation is brought about by the inhibitory action on myelopoiesis by the lymphoid substance. Normally, the two substances are in balance, but altered relationship of myeloid to lymphoid substance or lymphoid to myeloid substance underlies the leukemic state.

In 1940 Jones, Miller, and Hause²⁷ reported the treatment of 2 patients with subacute hypocytic lymphoblastic leukemia who were given daily injections of myeloid urine extracts and who were observed to have clinical, hematologic, and bone-marrow remissions for short periods. Wearn, in discussing this paper, stated that his group had attempted the same experiment, but could not confirm the findings. Miller, Herbut, and Jones,²⁸ in 1947, reported the use of crude "myelokentric acid" in the treatment of 8 cases of lymphoblastic leukemia. Thirteen partial clinical, hematologic, and bone-marrow remissions occurred following the use of the extract.

The implications of the above work and the therapeutic possibilities raised prompted us to study the problem. This report is concerned with the results obtained by the injection into guinea-pigs of extracts prepared from normal beef liver.

MATERIALS AND METHODS

Forty-nine guinea-pigs, 44 males and 5 females, were studied. The majority were young, ranging in weight from 200 to 400 gm. Occasionally, older animals up to 700 gm. were used. No attempt was made to secure a uniform strain. All animals were standardized to the laboratory environment and diet for a minimum of 1 week before the start of the experiment. Peripheral blood counts were made before the first injection and at least once a week during the course of the experiment. Animals were weighed at these times. The laboratory diet consisted of purina rabbit chow (in pellet form), greens, and water *ad libitum*. Animals were sacrificed by the intracardiac injection of 10 to 20 cc. of air. Body weights and wet weights of liver and spleen were recorded. Imprints of the cervical node, spleen, and bone marrow were taken routinely and stained with Wright's stain. Microscopic study included sections of the cervical nodes, thymus, axillary nodes when enlarged, lungs, heart, liver, spleen, adrenals,

kidneys, testes or ovaries, mesenteric nodes when enlarged, femoral and vertebral (lumbar) bone marrow, and tissue from the sites of injection. Hematoxylin and eosin stains were used routinely. Control animals were included with each series of animals tested.

Extracts of beef liver were prepared in the following manner. Dried beef liver was extracted with ethanol. The ethanol extract was concentrated, saponified, and extracted with ether to remove neutral materials. The alkaline solution was saturated with carbon dioxide and extracted with ether to remove phenolic materials, then acidified with hydrochloric acid and re-extracted with ether. The ether extract was evaporated to dryness and extracted with petroleum ether. The petroleum ether extract was extracted with methanol to remove benzoic materials and made into lead salts in hot alcoholic solution, which was then filtered. The alcohol insoluble lead salts were extracted with ether to remove ether-soluble salts. The ether insoluble lead salts were regenerated to acid form, dissolved in acetone, and kept at -20°C . for crystallization. The solution was then filtered to remove crystals of palmitic and/or stearic acids. The filtrate was concentrated and separated by succination into carbinols and noncarbinols. The B-acids fraction was the final filtrate before succinate separation. Each of the three fractions was suspended in cottonseed oil in the following manner: 1 cc. of B-acids extract suspension contained the equivalent of 435 gm. of original liver. One cc. of carbinol suspension contained the equivalent of 8,220 gm. of original liver. One cc. of noncarbinol suspension contained the equivalent of 591 gm. of liver.

Injections were made into alternate thigh muscles of the animals. The single dosages varied, and in most instances injections were given every second or third day. One series of animals was injected daily. Control animals received injections of the cottonseed oil.

DESCRIPTION OF PATHOLOGIC CHANGES

B-Acids (Hodgkin's-like) Reaction

In general, the reactions to B-acids observed in these animals were not striking. The gross pathologic changes were limited to the site of injection where the involved muscle was indurated and somewhat necrotic. Microscopically, the lesions consisted of foci of polymorphous and monocytoïd cells with reticulum hyperplasia and varying numbers of eosinophils. No giant cells were noted. In some areas fibroblasts and fibrous tissue were seen in and about these lesions. In the cervical nodes, the lesion usually was confined to the pulp; in 2 animals, monocytoïd cells in the centers of follicles were noted. In the liver, the lesions were periportal (Fig. 1). The splenic pulp was

most commonly involved. Myeloid hyperplasia of the bone marrow with many megakaryocytes was a frequent finding. The site of injection showed evidence of mild necrosis of muscle with little cellular infiltration and moderate fibrous reaction.

Carbinol (Lymphoid) Reaction

Gross examination of the animals injected with the carbinol fraction revealed enlargement of the cervical nodes and occasionally concomitant enlargement of the axillary and mesenteric lymph nodes. Only one animal in this series showed enlargement of the spleen. The lungs presented a white patchy or nodular appearance. The bone marrow was not remarkable. The muscles of the thigh were indurated and on cut section showed small areas of necrosis. Microscopic examination disclosed hyperplasia of the lymphoid elements of the cervical node and spleen (Fig. 2). In our work, capsular invasion was observed in only 2 animals. Periportal and, at times, intrasinusoidal foci of round cells were noted in the liver. Increased lymphoid tissue was present in the lungs. The kidneys frequently were involved, with intertubular round cell infiltration of the cortex and occasional lymphocytic foci in the submucosa of the pelvis. Foci of round cells in and about the medulla of the adrenal were seen but, in general, adrenal involvement was less common than in the noncarbinol-treated animals. The bone marrow was involved only infrequently. Injection sites revealed very little reaction to the extract. There was some necrosis of muscle fibers and fibrous tissue reaction. Cellular infiltration was almost completely lacking.

Noncarbinol (Myeloid) Reaction

In animals injected with the noncarbinol fraction, gross examination revealed moderate enlargement of the cervical nodes and adrenals, considerable enlargement of the spleen, and occasional gross hemorrhages into the femoral bone marrow. The thighs were markedly indurated and a few animals developed suppuration with purulent discharge. On section these areas revealed abscesses which were well walled-off. Microscopically, the lesions consisted of varying degrees of myeloid metaplasia, most pronounced in the spleen (Figs. 3 and 4), with frequent foci in the pulp of the cervical lymph nodes. The liver was diffusely involved with foci of immature myeloid cells. The lungs were either normal or showed a slight reduction in the amount of lymphoid tissue. The adrenals presented uniform changes, with foci of immature myeloid cells in the cortex which at times involved the medulla. Medullary hemorrhage was sometimes noted. Occasionally, the capsule of the organ was invaded (Fig. 5). Intertubular infiltration

of myeloid cells near the renal glomeruli was noted (Fig. 6). No infiltration of the testes occurred, but atrophy of the seminiferous tubules was present. Myeloid hyperplasia of the elements of the bone marrow was present in most cases. The site of injection showed considerable necrosis of muscle and surrounding tissue, with large numbers of segmented neutrophils and eosinophils infiltrating between the muscle fibers. Islands of suppuration with central necrosis and caseation were noted. In some cases fibroblastic reaction about these lesions was very marked.

OBSERVATIONS

B-Acids (Hodgkin's-like) Fraction

Nine young guinea-pigs were given three equally divided doses of the B-acids extract varying from 0.4 to 1.8 cc., and representing 177 to 780 gm. of original beef liver (Table I). The peripheral blood was within normal range in all subjects. The general condition of the animals remained good throughout. Animals were sacrificed or died at times varying from 0 to 60 days after the final injection. Seven animals showed evidence of Hodgkin's-like lesions in the cervical nodes, spleen, liver, adrenal, and kidney. The bone marrow revealed myeloid hyperplasia in 6 guinea-pigs, lymphoid hyperplasia in one, and no changes in 2. Myeloid metaplasia of the cervical nodes and spleen was noted in one guinea-pig. One animal was negative.

Carbinol (Lymphoid) Fraction

Seven male and 5 female guinea-pigs with initial weights varying from 285 to 483 gm. were used for carbinol injection (Table II). Four series of 3 animals each were given doses varying from 0.10 to 3.35 cc. of extract, representing 822 to 24,720 gm. of beef liver. There were no side reactions, and no deaths occurred during the course of the experiment. All of the animals remained in good clinical condition and weights were maintained during the injection period. Peripheral blood counts were normal. Animals were sacrificed from 10 to 147 days after the final injection. Definite evidence of a lymphoid reaction was present in 5 animals. Four others reacted to a lesser degree. Myeloid changes in the cervical nodes, spleen, and liver were noted in one animal while 2 others were negative.

Noncarbinol (Myeloid) Fraction

A total of 18 guinea-pigs was used for injection of the noncarbinol fraction (Table III). In the first experiment, 12 males varying in weight from 263 to 391 gm. were given divided doses every third day totaling 1 to 4 cc. This represented 591 to 2,364 gm. of beef liver.

TABLE I
Results of Injection of the B-Acids (Hodgkin's-like) Fraction

Guinea-pig no.	Initial weight gm.	Extract, total dose cc.	Equivalent liver gm.	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection days	Last injection days		Initial millions	Terminal millions	Initial thousands	Terminal thousands	
1	265	0.4	177.3	16	10	S	5.03	5.44	12.9	15.8	±H
2	255	0.4	177.3	64	58	S	5.13	4.6	10.2	8.8	±H
3	270	0.9	390	16	10	S	5.3	5.07	12.3	23.1	±H
4	312	0.9	390	66	60	S	4.53	5.1	9.3	8.1	±H
5	305	0.8	347	6	0	D	4.62	5.02	13.2	13.4	None
6	345	1.2	520	66	60	S	5.41	4.4	9.8	10.3	±H
7	283	1.5	665	8	2	D	6.17	5.95	10.3	18.2	±H
9	242	1.8	780	66	60	S	6.08	5.0	7.4	11.4	±M
10	300	1.2	520	6	0	D	5.42	5.02	12.2	8.3	±H

Key: H = Hodgkin's-like reaction; M = myeloid reaction; S = sacrificed; D = died.

TABLE II
Results of Injection of the Carbinol (Lymphoid) Fraction

Guinea-pig no.	Initial weight gm.	Extract, total dose cc.	Equivalent liver gm.	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection days	Last injection days		Initial millions	Terminal millions	Initial thousands	Terminal thousands	
12	334	0.1	822	77	74	S	5.4	4.4	10.9	29.1	++L
13	285	0.1	822	80	77	S	5.6	4.4	8.3	7.0	±L
14	320	0.1	822	13	10	S	4.6	5.0	8.0	8.2	±L
15	285	0.15	1156	77	71	S	5.4	5.3	10.0	20.0	±L
16	357	0.15	1156	78	72	S	5.0	6.0	9.6	13.8	++L
17	363	0.15	1156	16	10	S	4.5	4.2	7.9	13.8	++L
18	343	0.2	1644	156	147	S	5.1	4.2	9.6	33.7	++M
19	295	0.2	1644	131	122	S	6.5	5.1	15.6	16.5	++L
20	322	0.2	1644	20	11	S	4.6	5.1	6.9	7.5	++L
36	483	3.35	24,720	80	26	S	5.4	5.1	11.5	12.5	None
37	440	3.35	24,720	80	26	S	4.3	4.0	9.9	11.2	++L
38	449	3.35	24,720	76	22	S	4.7	5.5	10.5	10.5	±L

Key: L = lymphoid reaction; M = myeloid reaction; S = sacrificed.

Injections were given intramuscularly into alternate hind limbs. It was found that a total dose of 2.5 cc. given in 0.5 cc. doses every third day produced the best response. Subsequently, 6 other males ranging from 425 to 652 gm. were placed on this regimen and the survivors were sacrificed 9 days after the final injection. During the experiment, anaphylactoid reactions to the injected extract were noted in 6 animals. Three of these died within 10 minutes of the injection. In this series, all subjects lost weight during the injection period and 2 died spontaneously. The others recovered rapidly and went on to gain fairly well. The general condition of the animals was fair. In all cases varying degrees of reaction to the extract were noted in the hind legs. Three animals showed ulceration of one or two toes of one hind foot and one showed similar changes in both hind feet. In 3 guinea-pigs moderate anemia with normoblasts and myelocytes in the peripheral smear was observed. The experiment was terminated for the surviving animals 9 to 104 days after the final injection. Eight animals showed definite myeloid reaction, and 5, less striking lesions. Five others presented a mixed myeloid and lymphoid stimulation in which the cervical nodes, pulmonary lymphoid tissue, and spleen were predominantly lymphoid or polymorphous in cellular type, while the infiltrations of the liver, kidneys, and occasionally the adrenals were of the immature myeloid type. In one animal, isolated foci of myeloid and lymphoid infiltration were noted in the kidney and adrenal. The bone marrow showed positive evidence of myeloid hyperplasia in 13 animals.

Cottonseed Oil Control

Seven male guinea-pigs weighing from 210 to 300 gm. were used as controls (Table IV). The animals were divided into two series of 4 and 3. The first series was injected intramuscularly in alternate hind limbs with cottonseed oil in 0.5 cc. doses every third day until a total dose of 3.5 cc. was given. The second series was given 0.5 cc. of the same oil daily for a total dose of 3.0 cc. There were no untoward reactions to the injections and the animals remained in excellent health throughout the experiment. During the injection period, the weights remained constant, but immediately thereafter normal weight gains were noted. The peripheral blood picture was always within normal limits. Animals were sacrificed from 10 to 97 days after the final injection. Gross pathologic changes were limited to moderate induration of the muscles at the sites of injection. Three animals had myeloid changes of varying degree. Two animals showed mild mixed myeloid and lymphoid infiltration. In them, the spleen showed evidence of myeloid metaplasia, while the adrenals and kidneys had

TABLE III
Results of Injection of the Noncarbinol (Myeloid) Fraction

Guinea-pig no.	Initial weight gm.	Extract, total dose cc.	Equivalent liver gm.	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection days	Last injection days		Initial millions	Terminal millions	Initial thousands	Terminal thousands	
25	305	1.0	591	22	13	S	5.2	4.4	8.1	14.0	± M
26	295	1.0	591	72	83	S	4.8	4.8	6.5	11.0	++ M
27	307	1.0	591	112	103	S	4.2	5.0	4.6	30.0	++ M & L
28	282	2.5	1477	21	9	S	4.6	3.2	5.6	16.2	++ M
29	294	2.5	1477	116	104	S	4.2	5.6	7.3	13.9	++ M
30	301	2.5	1477	98	86	S	4.5	6.3	5.8	19.3	++ M
31	333	3.5	2000	113	95	S	6.0	4.4	16.0	33	++ M
32	263	3.5	2000	112	94	S	6.0	5.2	10.0	8.4	++ M
33	307	3.5	2000	8	0	D	5.7	4.1	10.8	19.5	++ M & L
39	391	4.0	2364	76	52	S	6.1	5.0	8.7	7.8	++ M & L
40	365	4.0	2364	74	50	S	5.1	5.8	6.0	9.8	++ M & L
41	327	2.5	1477	12	0	D	5.4	5.2	12.0	6.0	++ M
64	462	2.0	1182	12	3	D	5.2	3.6	11.7	25.0	++ M
67	608	2.5	1477	21	9	S	5.2	5.1	13.8	15.3	++ M
69	645	2.5	1477	21	9	S	5.3	5.0	20.0	14.0	± M
70	425	2.5	1477	21	9	S	4.95	5.05	16.5	18.0	++ M
71	510	2.5	1477	18	6	D	4.9	3.2	12.0	18.5	++ M & L
72	652	1.5	896	9	3	D	5.3		8.9		± M & L

Key: M = myeloid reaction; L = lymphoid reaction; M & L = mixed reaction; S = sacrificed; D = died.

TABLE IV
Cottonseed Oil Control

Guinea-pig no.	Initial weight gm.	Extract, total dose cc.	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
			Initial injection days	Last injection days		Initial millions	Terminal millions	Initial thousands	Terminal thousands	
21	285	3.5	116	97	S	5.3	5.7	6.1	19.5	+ M & L
23	300	3.5	90	71	S	5.4	4.5	6.4	13.6	± M & L
24	292	3.5	90	71	S	4.5	4.7	6.3	9.0	++ M
34	265	3.5	116	97	S	4.8	4.7	14.0	11.4	± L
52	62	3.0	16	10	S	4.0	6.8	20.2	14.4	None
53	210	3.0	78	71	S	5.2	5.3	9.3	6.3	++ M
54	265	3.0	64	57	S	4.5	5.4	5.2	8.3	± M

Key: M = myeloid reaction; L = lymphoid reaction; M & L = mixed reaction; S = sacrificed.

lymphoid or mixed cellular infiltrates. A very mild lymphoid reaction was noted in one guinea-pig and another was negative. The bone marrow was negative in 5 animals while 2 showed some degree of myeloid hyperplasia.

DISCUSSION

The clinical course and the pathologic changes noted in the guinea-pigs in our study differ from those observed in spontaneous leukemia in animals and human patients. The pigs maintained good weights and were well clinically. Except for a small group in the noncarbinol (myeloid) series, no anemia or change in the white cells of the periph-

TABLE V

Frequency of Organ Involvement Following Use of the B-Acids (Hodgkin's-like) Fraction

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
±H	4	5	3	1	3		
H	1	1	1				
L	1						1
M	1	1					6
None	1	2	5	3	5	2	2
No sections	1			5	1	7	

Key: H = Hodgkin's-like reaction; L = lymphoid reaction; M = myeloid reaction.

TABLE VI

Frequency of Organ Involvement Following Use of the Carbinol (Lymphoid) Fraction

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
±L	5	2	3	4	1	2	4
L	4	3		2	5	3	2
M	1	2	1			1	
None	2	5	7	6	6	6	5

Key: M = Myeloid reaction; L = lymphoid reaction.

eral blood was noted. Three of these animals showed a fall in hemoglobin and red blood cells with the appearance of normoblasts and immature leukocytes in the peripheral blood. These animals showed relatively severe histopathologic changes. The frequency and severity of organ involvement are shown in Tables V, VI, VII, and VIII. Thus the B-acids (Hodgkin's-like) fraction most commonly produced lesions in the cervical nodes, spleen, and liver, and stimulation of myelopoietic tissue of the bone marrow. The lesions in animals treated with the carbinol (lymphoid) fraction were most frequently observed in the cervical nodes and spleen. In one-half of the series, the liver, adrenals, kidneys, and lung were involved. The bone marrow was slightly infiltrated with round cells in one-half of the group. The non-carbinol (myeloid) fraction caused lesions in the spleen, adrenals, kidneys, liver, cervical nodes, and lung, in the order given. Bone-

marrow myeloid hyperplasia was noted in 13 of the 17 marrows examined.

Each fraction studied produced the anticipated lesion indicating a specific response. One animal given B-acids fraction and one given a carbinol fraction showed an unexpected myeloid reaction. Five animals given the noncarbinol fraction responded with a mixed myeloid and lymphoid lesion. These 5 animals were among those receiving the larger doses. Our results tended to confirm the observations of Miller and his group and Heinle and his co-workers that the noncarbinol fraction produces a more striking response than do the other two fractions.

TABLE VII
Frequency of Organ Involvement Following Use of the Noncarbinol (Myeloid) Fraction

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
± M	3	4	5	3	3	3	6
M	6	9	7	7	8	3	7
± M & L		3		1	2	1	
M & L				3	3		
None	9	2	6	4	2	10	4

Key: M = myeloid reaction; M & L = mixed myeloid and lymphoid reaction.

TABLE VIII
Frequency of Organ Involvement: Normal Butyl Succinate Control

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
± L	1	1	1	1	1	2	
L	2	1					
None		1	2	2	2	1	3

Key: L = lymphoid reaction.

The reciprocal relationship of myeloid and lymphoid tissues, as suggested by Wiseman, Doan, and Erf, is confirmed by the reduction of lymphoid tissue in the lungs and of a hypoplasia of the lymphoid follicles in myeloid-stimulated animals. Similarly, the increase in lymphoid tissue in the lung, lymph nodes, and spleen emphasizes the potentiating effect of lymphoid-stimulating substance. This hypothesis receives further confirmation with studies of the sites of injection. In the animals receiving the myeloid substance, granulocytic reaction was very marked; whereas, in the lymphoid-stimulated group these areas showed almost complete absence of inflammatory cells.

The nature of the substances involved has not been elucidated by this study. In the carbinol (lymphoid) group there were no anaphylactoid deaths, whereas there were 6 such deaths (of a total of 18 animals) in the noncarbinol (myeloid) group. Since our extracts are

very crude, the presence of a protein contaminant is a most probable cause. However, the interesting possibility of a protein conjugate as suggested by Hirschmann *et al.*²⁴ is also tenable.

We believe that our studies show that extracts of beef liver contain some substances which stimulate lymphoid hyperplasia and infiltration when the "carbinol" type is used, and a myeloid hyperplasia and infiltration when the "noncarbinol" type is injected into guinea-pigs.

The need for purification and concentration of the active hematopoietic stimulators involved is recognized and efforts in that direction are being continued. The presence or absence of these factors in other organs is being studied also.

SUMMARY

By a method which is described briefly, hematopoietic stimulating substances can be extracted from beef liver. These can be separated into a lymphoid stimulating factor and a myeloid stimulating factor. When injected into guinea-pigs these produce lesions appropriate to these designations, but the results are clinically and pathologically dissimilar to spontaneous leukemia. A reciprocal relationship between myeloid and lymphoid tissues was again confirmed. Further purification and concentration of the stimulator factors is necessary.

Extracts from beef liver were prepared by Drs. Frank Stirn and E. C. Yen at the Lederle Laboratories, Pearl River, N.Y.

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DESCRIPTION OF PLATES

PLATE 135

FIG. 1. Periportal focus of polymorphous and monocytoïd cells in liver (B-acids fraction prepared from beef liver). Hematoxylin and eosin stain. $\times 500$.

FIG. 2. Lymphoid hyperplasia in spleen (carbinol fraction prepared from beef liver). Hematoxylin and eosin stain. $\times 500$.

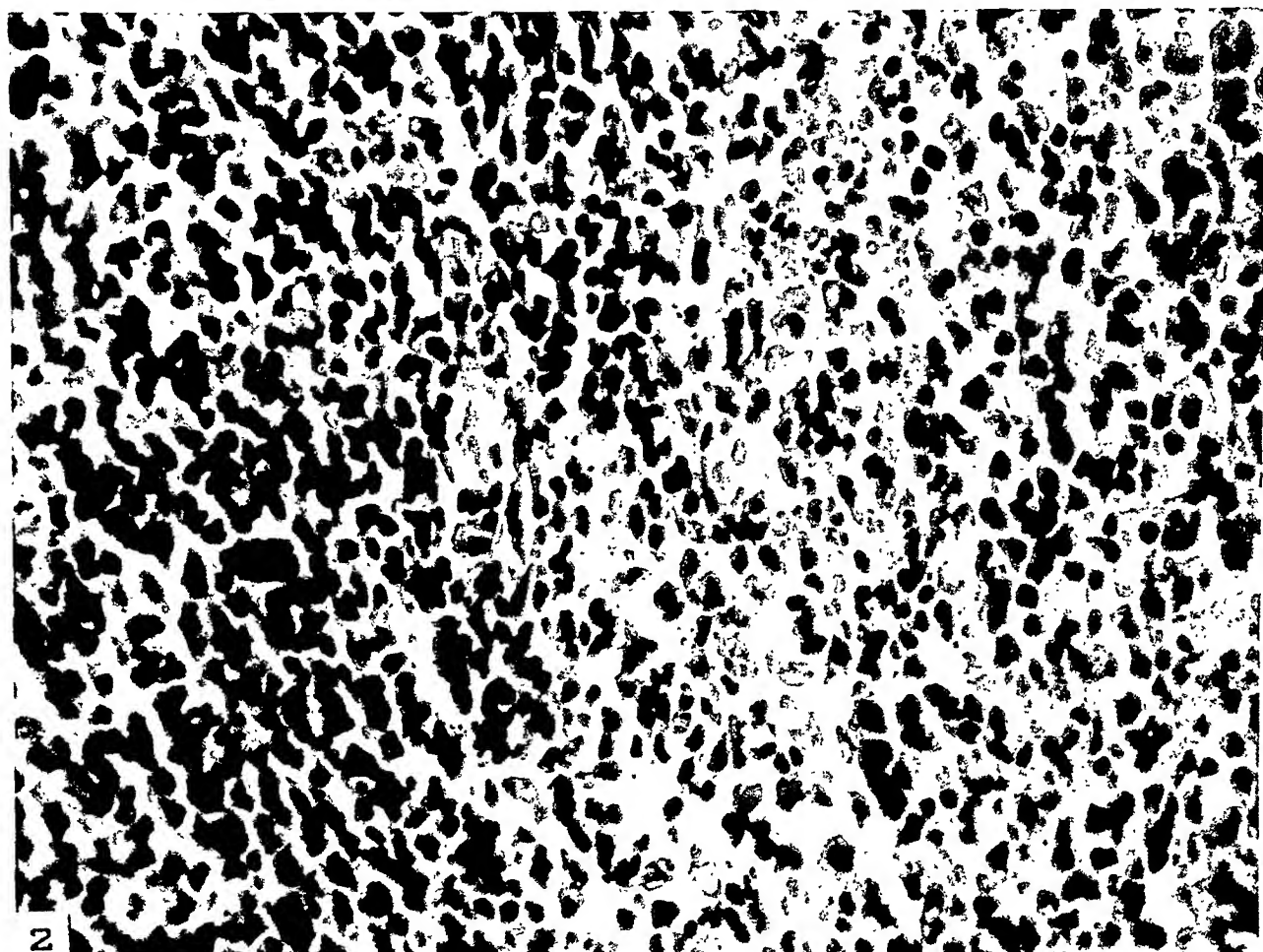
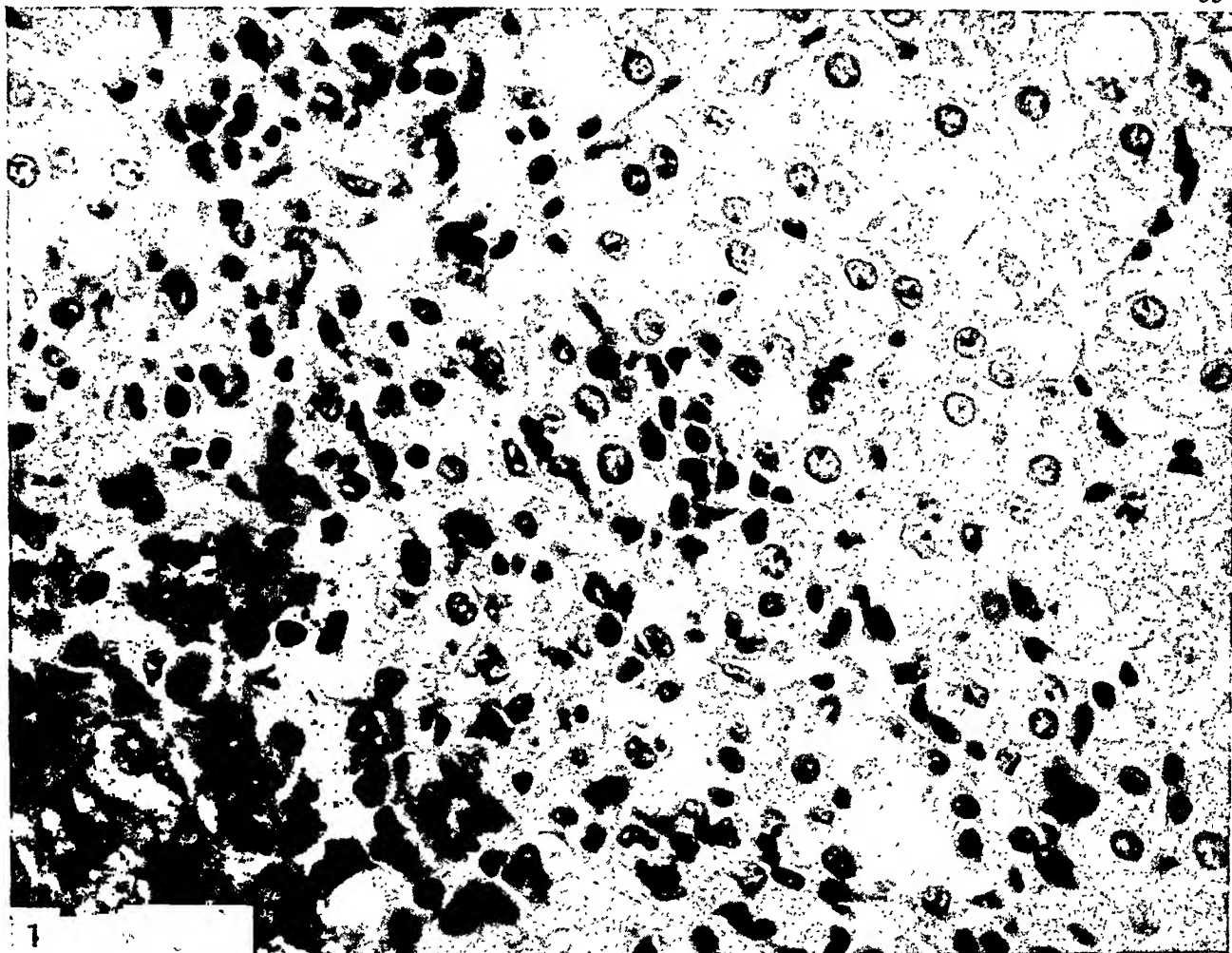


PLATE 136

FIG. 3. Myeloid metaplasia in spleen (noncarbinol fraction prepared from beef liver). Hematoxylin and eosin stain. $\times 100$.

FIG. 4. Myeloid metaplasia in spleen (noncarbinol fraction prepared from beef liver). Hematoxylin and eosin stain. $\times 500$.

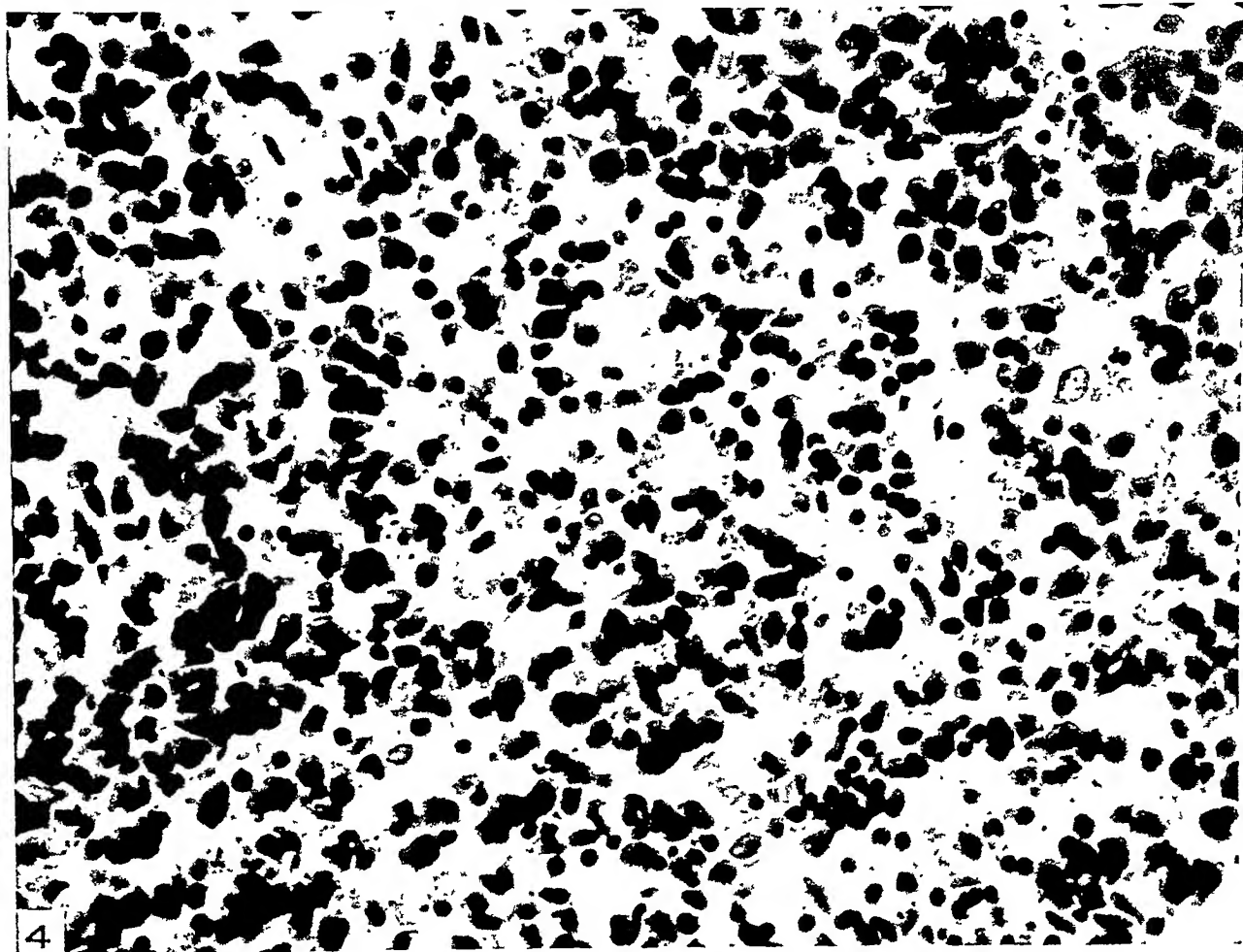
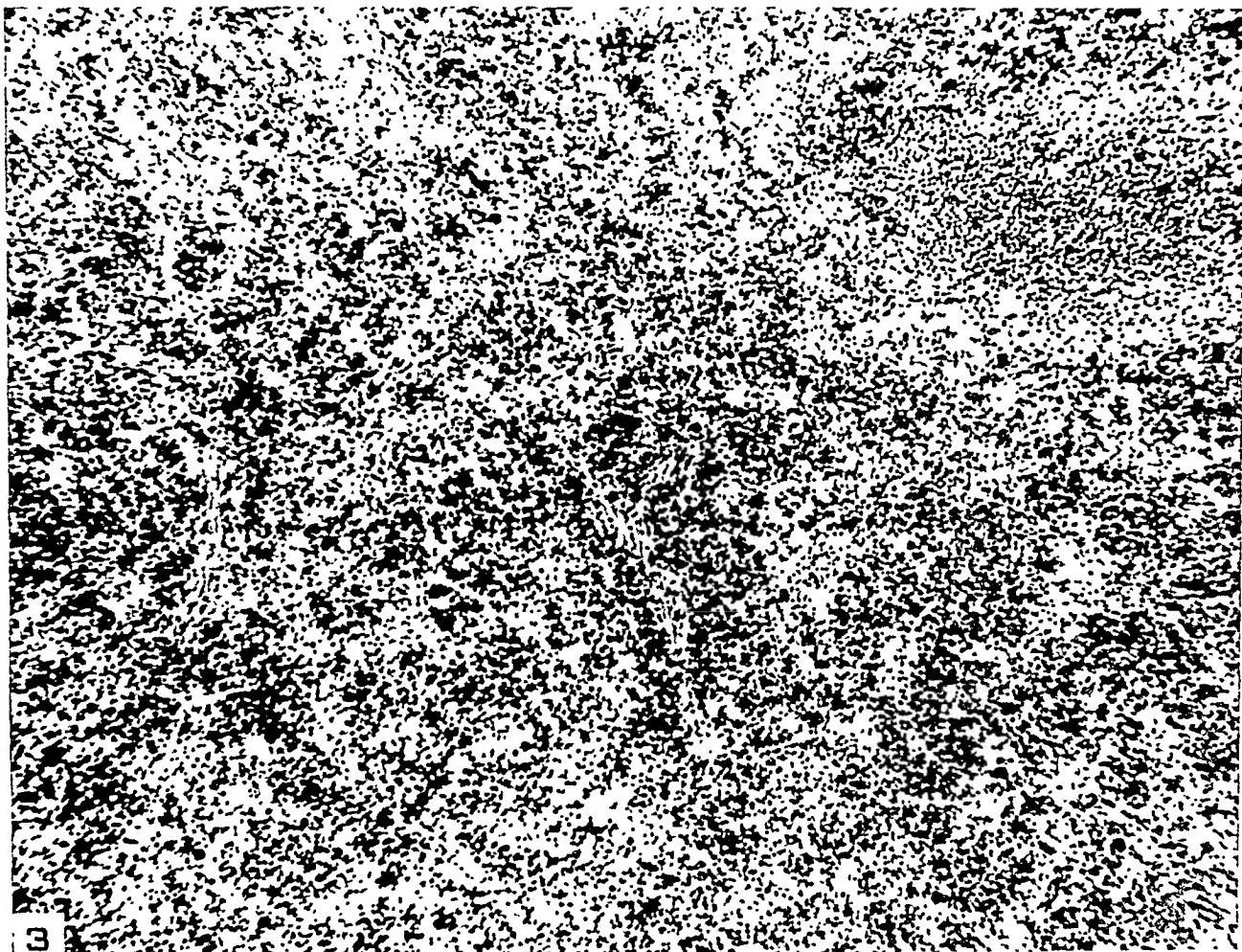
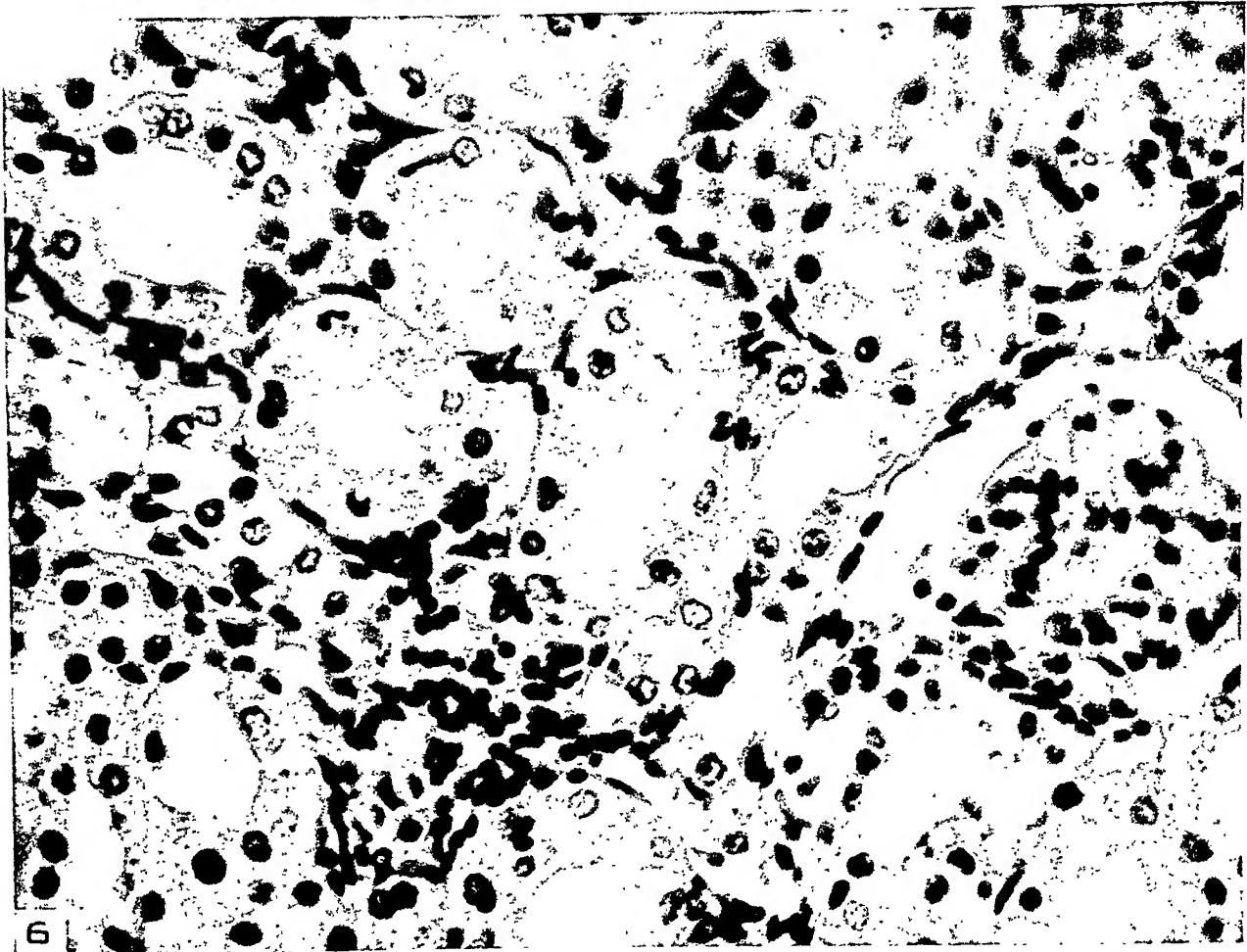
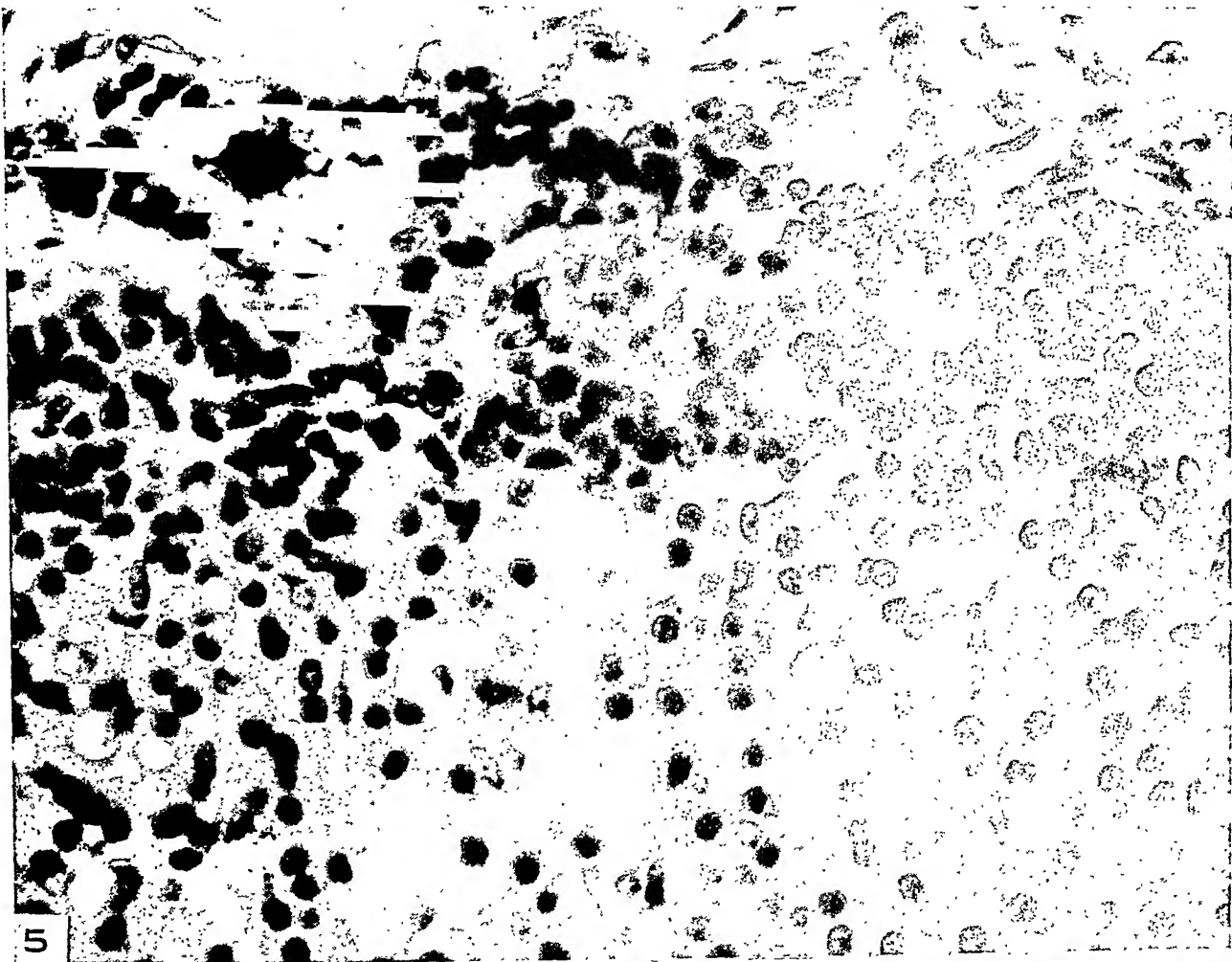


PLATE 137

FIG. 5. Myeloid infiltration in capsule of adrenal gland (noncarbinol fraction prepared from beef liver). Hematoxylin and eosin stain. $\times 500$.

FIG. 6. Intertubular infiltration of myeloid cells in kidney (noncarbinol fraction prepared from beef liver). Hematoxylin and eosin stain. $\times 500$.





THE PATHOLOGY OF GARGOYLISM

REPORT OF A CASE AND REVIEW OF THE LITERATURE *

LOTTE STRAUSS, M.D.†

(From the Laboratories of the Mount Sinai Hospital, Division of Pathology,
New York 29, N.Y.)

A syndrome of chondrodystrophic changes in the skeleton, corneal opacities, hepatosplenomegaly, and mental deficiency was reported by Hunter ¹ in 1917 and by Hurler ² in 1919. This is now well known as a clinical entity for which the term gargoylism was introduced by Ellis, Sheldon, and Capon ³ in 1936. More than 100 cases have been reported in the literature, and the clinical and radiographic features are well established. The syndrome is known to be familial; its genetic aspects have been discussed by Halperin and Curtis,⁴ de Rudder,⁵ and Böcker.⁶

The anatomic findings in gargoylism are still fragmentary and their interpretation is controversial. Most of the reports on autopsy findings have been incomplete, with main emphasis upon the examination of the cerebrospinal system. The lesions in the brain were reported to be identical with those in juvenile amaurotic idiocy.^{7,8} This led to the assumption that gargoylism is a lipid storage disease. Kressler and Aegerter ⁹ found vacuolated cells in many internal organs. Even though no lipid substances were demonstrated by histologic methods, Washington ¹⁰ defined the condition as "a disease of congenital origin characterized by chondrodystrophic changes in the skeleton and by a tendency toward the deposition of a lipid substance in the tissues, particularly in the brain," and coined the term lipochondrodystrophy. Schmidt ¹¹ described severe disturbances of endochondral ossification and demonstrated lipid granules in the cartilage cells. He considered the chondrodystrophic changes as an integral part of a disturbance of lipid metabolism.

In view of the small number of complete autopsy reports on record, the case of a 3-year-old girl with the characteristic history and clinical picture of gargoylism will be described.

REPORT OF CASE

E. L., a 3-year-old white girl of Polish extraction, was admitted to the Pediatric Service of the Mount Sinai Hospital on March 17, 1945. The patient's parents were fourth cousins. The patient had an older normal sister. The child was born by

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† Sarah Welt Fellow in Pathology.

spontaneous delivery after a normal full-term pregnancy. The birth weight was 4150 gm. She appeared to develop like a normal infant until the age of 6 months; however, she was unable to hold up her head. She did not sit up until she was 1 year old. The first tooth appeared at 1 year. When the child was about 6 months old the parents noted that she had a depressed nasal bridge and a chronic nasal discharge. When she was 1 year old a deformity of the head was noticed which progressed during the following 2 years. In addition there was flexion deformity of the fingers and limitation of motion of the extremities, as well as enlargement of the abdomen. Up to the age of 2 years mental and physical development were progressive though somewhat retarded. She was able to walk and talk, but from that time on development regressed, and at the time of admission she could not stand up, walk, or talk more than a few short words. There was progressive mental dullness. Her eyesight was poor. On the other hand, height and weight progressed normally. At the age of 3 years she weighed 15 kg.

Physical Examination. The patient was an obese, dull, irritable child with a large, deformed head and a purulent nasal discharge. She appeared mentally retarded and in poor contact with her environment. She did not raise her head or follow light. The skull was scaphocephalic; the fontanelles were closed. There were marked frontal and occipital protuberances. In the center of the frontal area there appeared to be an extra bone which was diamond-shaped and prominent. The eyes were widely spaced. Fundi could not be visualized because of bilateral, diffuse, punctate, corneal opacities. The nasal bridge was flat and the nostrils wide. The mouth was large, with thick lips (Fig. 1). The teeth were widely spaced and poorly developed, the lower teeth not yet fully erupted. The palate was high; the tongue appeared thick. The large head rested upon a short neck. Examination of the chest revealed rhonchi throughout both lungs. Respirations were 34 per minute. The heart appeared normal to percussion and auscultation. Pulse rate was 120; blood pressure, 120/80 mm. Hg. The abdomen was protuberant, and there was a small umbilical hernia. The liver and spleen were palpable 3 to 4 finger-breadths below the costal margins. The skin revealed abundant lanugo, especially over the back, forehead, and upper lids.

Fingers and toes were flexed and could not be fully extended; there was also limitation of extension of the knees and elbows. The arms could not be abducted above the head. The extremities appeared somewhat shorter than is normal. There was lumbar kyphosis. The neurologic status was normal.

A clinical diagnosis of lipochondrodystrophy (Hurler's disease) was made. Laboratory findings: Hemoglobin, 88 per cent; white blood cell count, 13,000, with 69 per cent polymorphonuclear cells, 27 per cent lymphocytes, 3 per cent monocytes, and 1 per cent eosinophils. The urine showed 3 plus albumin and one to three white cells in the sediment. Wassermann test of the blood was negative. Tuberculin patch test was negative. Serum phosphorus was 4.8 mg. per 100 cc.; serum calcium, 10.8 mg. per 100 cc.; blood cholesterol, 220 mg. per 100 cc. Electroencephalogram (Dr. H. Strauss) showed "severe diffuse cerebral dysfunction."

Roentgenologic Findings (Dr. W. H. Meritt). Examination of the skull showed the presence of a scaphocephalic deformity (Fig. 2). The frontal area bulged anteriorly. The frontal sinuses were absent. A small metopic suture was evident. There was also considerable enlargement of the posterior cranial fossa. The sella turcica was not well outlined, but appeared to be widened. The dorsum sellae appeared thinner than usual, and there was some pointing of the posterior clinoids. The tuberculum sellae and the anterior clinoids were obscured. There was an increase in the convolutional impressions in the parietal region. The dorsolumbar spine showed kyphosis in the lower dorsal and upper lumbar region. The body of the second lumbar vertebra showed a defect at its anterosuperior aspect with prominence or beaking of its antero-inferior aspect. This change was present, but to a lesser degree, in the subjacent vertebral body. The ribs showed a general increase

in breadth throughout the thorax. There was bilateral coxa valga. Both acetabular roofs were shallow. There were broadening and deformity in the proximal and distal metaphysis of the humerus, radius, and ulna bilaterally. The metacarpals appeared deformed at both ends and the proximal phalanges at their distal ends. "The changes described are consistent with the diagnosis of gargoylism, but present no features which would distinguish this condition roentgenologically from the usual type of osteochondrodystrophy."

Course. On the third day of hospitalization the patient's temperature, which had been 37° C. on admission, rose to 40° C., believed to be due to sinusitis. Sulfadiazine was given for 3 days, with no effect on the fever. The child at no time appeared dangerously ill, but at the end of 3 days of this febrile course she suddenly died. Death was believed to be due to sudden heart failure. Post-mortem examination (no. 13098) was performed by Dr. P. Gruenwald 11 hours after death.

Gross Examination

The body measured 84 cm. in length (normal average, 88 cm.*). Its external appearance conformed to that found on physical examination. Description of the internal organs will be restricted to the pertinent findings.

The heart weighed 82 gm. (normal average, 59 gm.). There was a slight, diffuse, whitish thickening of the epicardium, most marked on the anterior surface. The tricuspid and mitral valves showed nodules up to 3 mm. in size, with a smooth surface and fibrous consistency, at the insertion of the chordae. The chordae themselves were thick and short, and the usual web-like insertion was almost absent (Fig. 3). The left ventricle was markedly hypertrophied (Figs. 3 and 4); the myocardium, pale red and firm. There was slight thickening of the endocardium in the left ventricle. Marked bulging of the septum was present in the aortic outflow tract. The aortic and pulmonary valves appeared edematous and fleshy (Fig. 4). The aorta and its large branches were thickened. The intima was white with yellow plaques, and coarsely wrinkled (Fig. 4). The openings of the coronary arteries were slit-like, the arteries themselves being wide and grossly normal.

The combined weight of the lungs was 168 gm. (normal average, 168 gm.). The large bronchi on the right contained much viscid, yellow, mucopurulent exudate. The large branches of the pulmonary artery showed slight thickening of their walls. The hilar lymph nodes were swollen and reddened. The larynx was grossly normal.

The liver weighed 580 gm. (normal average, 418 gm.). Its surface was smooth and reddish yellow, and its consistency soft. On the cut surfaces the lobular markings were poorly defined. The gallbladder mucosa showed some yellow stippling.

The spleen weighed 78 gm. (normal average, 37 gm.). Except for its enlargement, it showed nothing unusual.

* The normal weights and measurements are taken from Coppoletta and Wolbach.¹²

Each kidney weighed 40 gm. (normal average, 48 gm.) and had a smooth, yellowish surface. There were petechiae in the right renal pelvis.

The tongue showed prominent, pale papillae. Its surface was dry and gray. There were no gross abnormalities of the esophagus, stomach, and intestine. The mesenteric lymph nodes were moderately firm and had a strikingly yellow color. There were no gross abnormalities of the thymus, pancreas, adrenals, peripheral lymph nodes, bladder, and internal genital organs.

On sagittal section the thoracic spine showed widening of the disks posteriorly. In the lumbar spine there were abnormally wide remnants of cartilage ventrally (Fig. 5). The ribs were broad and flat. The costochondral junctions were regular and not widened. The marrow of the left femur showed, on longitudinal section, a mottled appearance, due to alternating hematopoietic and fatty areas. The femur was slightly curved with the convexity directed laterally. The sagittal and lambdoid sutures of the skull had disappeared. The coronal suture was partly open. The fontanelles were closed. Traces of the frontal suture were seen externally. The base of the skull showed several irregular gyrus-like protrusions on the floor of the middle fossa, and marked protuberance of the petrous bone. The floor of the posterior fossa also showed protrusions, one of them at the posterior aspect of the foramen magnum, thus narrowing the foramen. There was marked flattening of the sphenoid bone.

Large portions of the liver, spleen, and brain were ground and preserved in cold acetone to await chemical examination.

Microscopic Examination

Heart. (Section was made through the posterior wall of the left ventricle, left atrium, and mitral valve.) The myocardium was hypertrophied. There were no vacuoles in the cytoplasm of the muscle fibers. Small foci were present where the muscle fibers were atrophied and encroached upon by delicate connective tissue septa. These were infiltrated by accumulations of large cells which were polygonal or oval; the cell body was vacuolated; the cytoplasm stained only very faintly with eosin and was finely granular or fibrillar. There was a small, dark, eccentric nucleus (Fig. 6). The cells usually were accompanied by varying amounts of collagen fibers. The connective tissue surrounding the small myocardial blood vessels had a similar appearance. Anitschkow cells could be encountered in these foci; their cytoplasm was poorly visible and appeared not to be vacuolated. Some

of the medium-sized branches of the coronary artery showed segmental thickening of the media, due to the presence of large, vacuolated, spindle-shaped cells; where these were present the smooth muscle fibers of the media were atrophic and replaced by connective tissue fibers. Where the large cells were missing, the muscle fibers in the media were well preserved. The intima and the internal elastica were intact. Vacuolated cells were seen also in the adventitia.

The endocardium of the atrium was diffusely thickened (Fig. 7), and there was focal thickening of the ventricular endocardium due to the presence of swollen cells accompanied by an increase of collagen fibers. No change in the elastic fibers was seen. The endocardial thickening was most marked at the base of the ventricle, in the region of the mitral ring, and continued throughout the mitral valve, the thickness of which was increased to about three times the normal (Fig. 7). It was made up of large numbers of swollen, vacuolated cells (Fig. 8). The cell body varied from a plump spindle to polygonal shape, apparently determined by the environment of the individual cell. Often the cells were arranged in rows or columns of varying length, either pressing upon each other or isolated and completely surrounded by a ground substance. Their cytoplasm stained only very faintly; most of it remained unstained. With Mallory's phosphotungstic acid hematoxylin stain, extremely delicate granules could be visualized in the cytoplasm. Most of the cells had a small, dark, eccentrically located nucleus. A considerable number, however, possessed nuclei exhibiting the characteristic appearance of the nucleus of the Anitschkow cell. Here, these cells had abundant cytoplasm showing evidence of storage, and well defined cell borders. Wherever these large cells were present there was also a marked increase in collagen fibers (Fig. 8), forming wavy bands of varying thickness throughout the valve. The vacuolated cells failed to stain by Best's carmine method for glycogen or by the sudan III and Smith-Dietrich stains for lipoid.

Aorta. The aorta was markedly thickened (Fig. 9). This was due chiefly to the width of the intima which exceeded that of the media. The thickening was patchy and did not affect the entire circumference. The microscopic appearance of the intima resembled that of the mitral valve. It consisted of layers of swollen, spindle-shaped, vacuolated cells and fine wavy fibers which stained like collagen. (There was a scanty cement substance staining blue with Mallory's aniline blue method.) In the media there were multiple spindle-shaped, clear cells between the muscle and elastic fibers. They had a slightly eccentric nucleus and probably were cells of the same kind as seen in the intima.

Frozen sections of the aorta stained with sudan III, Nile blue sulfate, and by the Ciaccio and Smith-Dietrich methods gave negative results. There was no doubly refractile substance within the cells.

Carotid Artery. The intima and media of a carotid artery included many vacuolated cells separated by wavy fibers. The artery was markedly thickened.

Mesenteric Artery. Section through a branch of a mesenteric artery showed marked thickening of the intima and media due to the presence of foam cells (Fig. 10).

Lungs. The lungs showed edema, emphysema, focal atelectasis, and acute bronchitis with plugging of bronchi with mucus and epithelial cells. There was focal hemorrhage. The arteries showed thickening of the media and intimal patches due to the presence of vacuolated cells. No foam cells were present in the parenchyma proper. The bronchial cartilage was normal.

Trachea. The trachea showed acute inflammation. Vacuolated cells were present in the pericartilaginous tissue.

Liver. The liver cells were diffusely vacuolated and their cytoplasm had a finely foamy structure (Fig. 11). The nuclei were normal. Best's carmine stain for glycogen was negative. Frozen sections stained with sudan III showed a number of large globules taking the stain, while the fine vacuoles remained unstained. A similar result was obtained with Nile blue sulfate (the fat globules stained pink). The Smith-Dietrich stain for lipoid was negative. No doubly refractile substance could be demonstrated. The Kupffer cells were swollen and finely vacuolated like the liver cells. No foam cells were present in the periportal spaces. The liver architecture was normal. There was no increase in connective tissue.

Spleen. The cells lining the splenic sinusoids were enlarged and their cytoplasm was finely honeycombed (Fig. 12). The malpighian follicles were small and depleted. Under oil immersion they revealed multiple vacuoles. There were no foam cells in the intersinusoidal reticulum or in the lymphatic tissue. The arterioles were not remarkable while in the walls of larger arteries vacuolated cells were seen. Sudan and Smith-Dietrich stains for lipoid were negative.

Pancreas. In the connective tissue around the larger pancreatic ducts, and in the peripancreatic tissue and arteries, vacuolated cells were found occasionally.

Kidney. In the interstitial connective tissue of the kidney, especially about vessels, there were scattered vacuolated cells. A large artery showed focal intimal thickening.

Alimentary Tract. In the esophagus there were hyperemia and focal

round-cell infiltration in the submucosa. The ganglion cells in the muscularis appeared vacuolated. In sections of the pylorus and of the small and large intestine, the cells of the myenteric plexus appeared enlarged and vacuolated. The submucous plexus of the intestine showed less conspicuous changes. In the muscularis propria of the small intestine there were many scattered, large, vacuolated cells. There was no increase in connective tissue.

Lymphatic Tissue. Section of a tonsil showed subacute inflammation. In the peritonsillar connective tissue there was a small accumulation of large, clear, vacuolated cells. The mesenteric and peripancreatic lymph nodes showed considerable post-mortem change. There were dilatation of the sinusoids and depletion of the lymphoid tissue. No vacuoles were seen in lymphocytes and reticulum cells.

Thymus. Large polygonal cells with vacuolated cytoplasm, having the appearance of histiocytes, were scattered through the thymic tissue.

Endocrine Organs. The adrenal cortex was considerably depleted of lipid. In the connective tissue occasional foam cells were noted. The ovary showed primordial and growing follicles. An occasional small group of foam cells was present in the stroma. One ovary showed a nodule of heterotopic adrenal cortex. The thyroid and pituitary glands showed no abnormalities.

Cornea. Immediately beneath the corneal epithelium there were elongated cells with their long axes parallel to the surface of the cornea (Fig. 13). These were larger than the basal cells of the corneal epithelium, and separated from it by a fine basement membrane. They appeared swollen, and in places produced a bulge in the lower surface of the corneal epithelium. Their cytoplasm stained very faintly with eosin which left irregular unstained portions, particularly near the nucleus, which was small, poor in chromatin, and somewhat eccentric. These cells did not form a continuous layer, but occurred in small islands. Where they were present, Bowman's layer could not be recognized. They appeared to derive from the fibroblasts of the substantia propria of the cornea. The latter was otherwise normal. There were no changes in Descemet's membrane or in the corneal endothelium.

Rib. The resting cartilage of the epiphysis of the rib was normal. The zone of proliferating cartilage was shortened and the cartilage columns were plump, and in places missing. No lipid substance could be demonstrated in the cartilage cells by sudan III or Smith-Dietrich stains. Where the proliferating cartilage cells were absent, there was an area of vascular connective tissue containing many large, swollen, vacuolated cells similar to those seen elsewhere in the connective tissues (Fig. 14). This area lay approximately in the center of the

epiphyseal zone, close to the costochondral junction. At its base there was a thin, transversely disposed layer of newly formed bone which lacked a calcified cartilage ground substance, in contrast to bony trabeculae normally formed from the cartilage. In places where short cartilage columns were present, the newly formed bony trabeculae were likewise short; however, they disposed themselves in the long axis of the bone and formed about a calcified cartilage matrix. The cancellous portion of the rib showed normal trabeculae. In the periosteum and perichondrium the collagen fibers appeared separated by large numbers of vacuolated, spindle-shaped cells having small eccentric nuclei. The periosteum was markedly thickened by the presence of the large cells and a conspicuous increase in the number of collagen fibers (Fig. 15). The infiltration with vacuolated cells was most striking in the cambium layer of the periosteum. The infiltrated periosteal tissue appeared to be engaged in the resorption and reconstruction of cortical bone which, along the surface of contact, showed many shallow and deep lacunae filled with the swollen cells (Fig. 15). Occasionally, small fragments of newly formed bone were completely embedded in the infiltrated periosteal tissue, and the osteocytes still resembled the swollen periosteal cells. It thus appears that bone may be formed directly within this tissue. The cortex itself was thick. The endosteum was thin and delicate, and there were very few osteoblasts and osteoclasts. The perichondrium also showed infiltration with large cells. These appeared to become incorporated in the cartilage, which was more cellular in its peripheral portions than in its depth. Also, the cartilage cells there appeared somewhat larger, but otherwise were not remarkable. One of the ribs showed a circular periosteal band ("Perioststreifen") interposed between the epiphyseal cartilage and the metaphysis, thus producing a marked constriction of the epiphyseal zone. The peripheral parts of the shaft were formed directly from the periosteal band instead of from cartilage. The same foam cells present elsewhere in the periosteum were scattered throughout the periosteal wedge, which otherwise was formed by dense collagen fibers.

Lumbar Vertebra. Sagittal section of the lumbar vertebra showed the structure of the spongiosa to be normal. The marrow was cellular and there were few fat cells. Proliferating cartilage was very scanty; columns of cartilage either were missing (Fig. 16) or very short (Fig. 17). Thus, much of the cartilage which would normally proliferate had remained in the resting stage (Fig. 16). Penetration of marrow cavities into the cartilage was very slight and occurred only where the short columns were present (in the center of the epiphyseal line). The zone of provisional calcification was almost absent, and bone was laid

down directly at the border of the epiphyseal cartilage, as a transverse layer (Fig. 17). Accordingly, ossification was mainly perichondral. The longitudinally directed trabeculae seen in normal bone were rare.

Along the anterior border of the vertebra there was a rather deep groove producing a concave outline of the vertebral body. This groove was filled with thick collagen fibers forming interlacing bundles. Adjacent to the cortex there was a layer of vacuolated cells which appeared to penetrate into the cortical bone, giving it an eroded border. Very few osteoclasts were seen, but the vacuolated cells appeared to exert osteoclastic activity. Small fragments of bone were laid down in the infiltrated zone of the periosteum.

Another lumbar vertebra showed a marked overgrowth of cartilage along its anterior border. Apparently this was the result of the poor endochondral bone formation from the epiphyseal cartilage. Thus the spongiosa of the vertebra became disproportionately small and its outline deformed. The anterior border of the vertebra became shorter than the posterior border and much more concave. The concavity was filled with periosteal and perichondral tissue consisting of interlacing fibers which blended with the overhanging cartilage, and of numerous vacuolated cells which appeared to become incorporated in the cartilage.

Head of the Femur and Sternum. The changes seen in the head of the femur and sternum were comparable to those seen in the ribs and vertebral bodies.

Skull. The skull was thick and compact. There was no diploe. The haversian systems were well developed. The periosteum showed infiltration by vacuolated cells in the cambium layer.

Central Nervous System. The gross and microscopic findings in the brain and spinal cord will be reported in detail elsewhere.¹³ There was a marked internal hydrocephalus. On microscopic examination generalized degeneration of ganglion cells with ballooning and loss of Nissl substance was found. This change was most marked in the cerebral cortex and in the anterior horns in the spinal cord. There was a moderate increase in glial elements in the cortex. The swollen ganglion cells stained deeply with sudan III.

DISCUSSION AND REVIEW OF THE LITERATURE

Ellis, Sheldon, and Capon³ introduced the term gargoylism because the large head and inhuman facies common to the majority of patients affected with this condition reminded them of the gargoyles seen on some Gothic cathedrals. Although the syndrome seems to have been recognized as a condition *sui generis* as early as 1908 (Henderson¹⁴),

it did not appear in the literature until 1917 when Hunter¹ described the disease in two brothers. The syndrome then returned to the literature under various names, among which Hurler's disease, dysostosis multiplex, gargoylism, chondro-osteo-dystrophy, and lipochondrodystrophy are the most common.

The multiplicity of names and the variety of forms have made it very difficult to evaluate accurately the number of cases so far reported, and different figures are given by the reviewers. Henderson¹⁴

TABLE I

Reported Cases of Gargoylism in Addition to Those Listed by Henderson¹⁴ and Ellis¹⁵

Year	Author	No. of cases
1935	Reilly ¹⁶	3
1939	Berliner ¹⁷	3
1939	Nissler ¹⁸	1
1939	Höra ¹⁹	1
1940	Waardenburg ²⁰	2
1941	Stoeckel ²¹	2
1941	Ross, Hawke, and Brown ²²	4
1941	Veasey ²³	1
1942	Kny ²⁴	1
1942	Schmidt ¹¹	1
1942	Wolff ²⁵	1
1942	De Lange ²⁶	1
1942	Halperin and Curtis ⁴	1
1942	Harvey ²⁷	1
1942	Cordes and Hogan ²⁸	5
1943	Larson and Lichty ²⁹	3
1943	Lahdensuu ³⁰	4
1943	Rojas Dominguez ³¹	1
1943	Expósito Martinez and de Feria ³²	2
1943	Boldt ³³	1
1943	Böcker ⁶	1
1944	Lurie and Levy ³⁴	2
1945	Sear and Maddox ³⁵	1
1946	Debré, Marie, and Thieffry ³⁶	3
1946	Brouwer-Frommann ³⁷	1
1937	Bouman*	1
1940	Westrienen*	1
1946	Nja ³⁸	6

* Cited by Brouwer-Frommann.³⁷

collected 57 cases from the literature, and he and Ellis¹⁵ added 6 more. Since then, additional case reports have been published in various countries, and some previously reported cases have come to my attention which are not included in Henderson's paper. They are enumerated briefly in Table I.

This brings the total of known cases, including the one here presented, to 119. The case reports have illustrated amply the clinical features of the condition, which return with striking regularity in the majority of cases. The typical roentgenologic changes of the skeleton have been reviewed by Gillespie and Siegling,³⁰ Harvey,²⁷ and by Lar-

son and Lichty.²⁹ The occurrence of Sprengel's deformity has been stressed by Engel.⁴⁰

The familial nature of the condition has long been recognized. It has been encountered repeatedly in two or more siblings.^{4, 6} Consanguinity of the parents or grandparents of affected children has been found occasionally. The parents usually are healthy. However, in some reports deformities of the head, chest, or hands of one of the parents have been mentioned. An uncle of the patient reported by Jewesbury and Spence⁴¹ had a similar disease leading to early death. Slot and Burgess⁴² reported that a maternal aunt of their patient died in childhood as a deaf-and-dumb cripple. A suggestive history of mental deficiency in the mother's family was given by Lahdensuu.³⁰ Njå³⁸ studied the pedigree of a family in which 5 cases of gargoylism occurred. He observed that the afflicted members of the family were all males in whom the trait must have been transmitted from an unaffected mother. On this basis inheritance of a sex-linked type was suggested.

The few reports in the literature of autopsy findings in cases of gargoylism are gathered in Table II. Several of these cases were studied only incompletely, and in some the diagnosis of gargoylism must be considered as questionable.

Stoeckel's case²¹ was clinically a typical case of gargoylism. Unfortunately, the autopsy report is limited to a macroscopic description of only a part of the viscera. The case of "typus E" described by de Lange and co-workers⁴⁶ is included in Table II notwithstanding the negative findings in the brain. Its relation to gargoylism will be discussed below.

Cerebral changes similar to those seen in the juvenile form of amaurotic idiocy were confirmed by the reports of Ashby, Stewart, and Watkin,⁸ Kressler and Aegerter,⁹ Kny,²⁴ and de Lange.²⁶ Such changes in the nervous system were found in the present case; they will be reported in a separate publication by Green.¹³ As a result of this similarity, cases of gargoylism may have been interpreted erroneously as instances of amaurotic idiocy. This occurred in the report of Zierl⁴³ who described Hurler's case along with 2 cases of amaurotic idiocy, stressing the presence of bone changes in all 3.

The interpretation of the changes in the central nervous system is of great importance for the understanding of gargoylism. Changes similar to those in juvenile amaurotic idiocy are not limited to a small group of closely related diseases, but constitute a more widely occurring type of nerve cell alteration than is commonly thought. This view was expressed by Jervis⁴⁷ when he described a familial syndrome which had clinical features in common with both gargoylism and

TABLE II
Reported Autopsy Findings in Cases of Gargoylism

Author	Sex, age	Clinical course	Heart, aorta	Spleen	Liver	Skeleton	Brain	Cornea	Other organs
Zierl, ⁴³ Tuthill ⁷	M 7 yrs.	Mental deficiency, deafness	Heart dilated	Enlarged	Enlarged	Chondrodystrophy; osteophytes at base of skull	Hydrocephalus; lipid granules in swollen nerve cells	Cloudy†	*
Reilly ¹⁶	M 10 yrs.	Normal mentality, deafness, speech defect; sudden death	Mitral stenosis, dilated right heart	590 gm.; dilated sinusoids	Enlarged; mild interlobular fibrosis	Sella small, shallow; dolichoccephaly	Post-mortem changes	*	Focal degeneration and necrosis in pituitary body, interlobular fibrosis in thyroid
Ashby, Stewart, and Watkin ⁸ (case 1)	M 19 yrs.	Mental deficiency; dwarfism; sudden death	Heart small†	100 gm.†	960 gm.; slight fatty change†	Thick skull; no diploë, wide sellar†	1046 gm.; unilateral hydrocephalus; nerve cells as in juvenile amaurotic idiocy	Cloudy†	Pituitary body and thyroid large; thyroid of fetal structure; other organs normal
Ashby, Stewart, and Watkin ⁸ (case 2)	F 9 yrs.	Large head; death in heart failure; sibling similar	Mitral stenosis, hypertrophic left ventricle	Normal	567 gm.; small, firm, congested; mild fatty change; no foam cells	Brachycephaly; frontal and temporal bossing; sellar enlarged†	1077 gm.; similar to preceding case; changes most marked in thalamus; focal gliosis	*	Thyroid large, with fibrosis and atrophy; kidneys normal; other organs*
Kressler and Aegerter ⁹	M 8 yrs.	Mental retardation; dyspnea, cyanosis; death in heart failure	Heart, 220 gm.; thick mitral, tricuspid valves; vacuoles in myocardial fibers; aorta streaked	140 gm.; vacuoles in some lymphocytes and reticulum cells; walls of small vessels infiltrated	1190 gm.; liver cells, cells in portal areas vacuolated	Sella shallow; clavicle short; acetabulum shallow; microscopically normal	1200 gm.; nerve cells large, lipid granules; degeneration of Nissl bodies	Cloudy; microscopically normal	Vacuolated cells in lungs, lymph nodes, testes; pituitary body enlarged, chromophobes "invaded;" thyroid, thymus normal

Berliner ¹⁷	M 6 yrs.	Died after operation for umbilical hernia	*	*	*	*	*	Cloudy; vacuolated cells	*
Höra ¹⁹	New-born		*	*	*	*	Acrocephaly; hypoplastic chondrodystrophy; radio-ulnar synostosis	Cloudy†	*
Stöckel ²¹ (case 2)	F 4 yrs.	Large liver; death from bronchitis	"Chronic endocarditis"†	Hyperplasia, large follicles†	*	*	Scaphocephaly; wide sella; elevations at base of skull†	Cloudy†	Coloboma of iris
Kny ²¹	F 6 yrs.	Typical syndrome	Fat in myocardium	Slightly enlarged; microscopically normal	Enlarged; early cirrhosis; liver cells vacuolated; Kupffer cells negative	Hydrocephalus; phyttes at base; sella wide; hypoplastic chondrodystrophy	Skull thin; osteophytes at base; sella wide; hypoplastic chondrodystrophy	*	Pituitary body slightly enlarged; nests of large, clear cells in thymus; fat in convoluted tubules in kidneys
Schmidt ¹¹	4 yrs.	*	*	*	*	*	Chondrodystrophy; lipid granules in cartilage cells	*	*
Rochat ¹¹	F 6 yrs.	Typical syndrome	*	*	*	*	*	Vacuolated cells	*
Wolf ²⁵	M 28 yrs.	Deafness; death in heart failure	"Chronic endocarditis of mitral, tricuspid and aortic valves"	Splenectomy at 10 years	Liver cells large, variable in shape	Calvarium thick; sella wide; external auditory canals narrow; mastoid antra small	Normal	*	Persistent thymus (7 gm.); exhaustion of lymph nodes

TABLE II (cont'd.)

Author	Sex, age	Clinical course	Heart, aorta	Spleen	Liver	Skeleton	Brain	Cornea	Other organs
De Lange, ²⁰ Zeeman ¹⁵	F 6 yrs.	Typical syndrome; unexpected death	Heart (80 gm.) and large vessels normal	78 gm.; normal	560 gm.; liver cells vacuolated; Kupffer cells swollen; focal fibrosis	Thin skull; lumbar kyphosis†	Internal hydrocephalus; changes as in amaurotic idiocy	Vacuolated cells	Lymph nodes swollen; degenerative changes in pituitary body; other endocrine organs normal; bronchitis
De Lange, Gerlings, de Kleyn, and Lettinga ¹⁶	M 19 yrs.	Hearing defect; systolic murmur; stridor; sudden death	Thick valves, "chondroid change"; acute myocarditis	405 gm.; slight fibroadenia	1720 gm.; slight cirrhosis; liver cells foamy, contained much glycogen	Scaphocephaly; flexion deformity; "chondroid change" in perichondrium; retarded bone growth; poor pneumatization of mastoid	Normal	Not cloudy†	Larynx narrow; "chondroid change" in perichondrium of larynx, trachea, bronchi; increase of connective tissue in pituitary body
Nja ³³	M 11 yrs.	Typical syndrome; chronic bronchitis; death in cyanosis and dyspnea	Heart, 140 gm.; hypertrophy of left ventricle; thick aortic and mitral valves; plaques in aorta	215 gm.†	1280 gm.†	Thick skull; protrusions at base of skull; narrow marrow cavities; long bones short, thick†	Internal hydrocephalus; thick leptomeninges†	Not cloudy†	Thymus, 41 gm.; bronchitis; massive lymphocytic infiltration of digestive and respiratory tracts
Present case	F 3 yrs.	Mentally retarded; death in sudden heart failure	Heart, 82 gm.; vacuolated cells in valves, endocardium, connective tissue, vessels of heart, and in aorta	78 gm.; vacuolated cells lining sinusoids	580 gm.; vacuolated liver cells and Kupffer cells	Scaphocephaly; protrusions at base of skull; chondrodys trophy; vacuolated cells in perosteum, perichondrium	Hydrocephalus; ballooning, degeneration of ganglion cells with sudanophil granules	Cloudy; vacuolated cells	Vacuolated cells in blood vessels; bronchitis; fat in convoluted tubules of kidneys; other organs normal

* Not examined or not reported.

† Not examined histologically.

‡ The other organs of this case were examined by Dr. Eugene Opie, who found microscopic changes almost identical with those described in the case here reported.

juvenile amaurotic idiocy (early onset of mental retardation, dwarfism, characteristic "gargoyle" facies, and thickening of the skull); however, other stigmata of gargoylism such as hepatosplenomegaly and corneal clouding were not present. At autopsy, examination of the liver and spleen revealed normal organs while the changes in the brain were found to be identical with those in well authenticated cases of gargoylism as well as in amaurotic family idiocy. Chemical examination of the brain disclosed the presence of neuramic acid which thus far has been found only in Tay-Sachs' disease (Klenk ^{48, 49}). On the basis of chemical differentiation, this case is to be grouped with the neuramic acid lipidoses while it is hard to classify it on the basis of clinical or morphologic characteristics. Tropp ⁵⁰ offered an explanation for the degenerative changes in the brain in the infantile form of Gaucher's disease which, modified, might be applied to gargoylism. He assumed that the degenerative changes in the central nervous system are secondary to the general metabolic disorder rather than an essential part of it in the sense of Spielmeyer.⁵¹ The finding of almost identical nerve cell changes in a variety of apparently nonrelated conditions (infantile morbus Gaucher, Niemann-Pick's disease, amaurotic idiocy, gargoylism) raises the question to what extent the lesions of the central nervous system are specific, and whether we are justified in placing such diverse diseases in a common group merely because of the morphologically similar changes in the brain. One may rather assume that swelling of nerve cells and the appearance in them of fat-like substances indicate a local disturbance of cellular metabolism resulting either from a deficiency of the necessary building materials (this deficiency being the direct result of a systemic metabolic disorder) or from an inherent inability of the nerve cells to utilize the substances necessary for their growth and preservation. This viewpoint was expressed by Globus ⁵² when he discussed the relationship of the lesions of the central nervous system in the various forms of amaurotic idiocy and Niemann-Pick's disease. The histologic findings in gargoylism seem to justify this assumption. In this disease only the swollen ganglion cells contain granules stainable with sudan while the storing cells elsewhere in the body cannot be stained with fat stains.

Internal hydrocephalus was found in the present case as well as in 5 other cases listed in Table II. In the case here presented, bony elevations were observed at the base of the skull which compressed the hindbrain and may have interfered with the drainage of liquor. In 4 of the other 5 cases in which hydrocephalus was found at autopsy, elevations or osteophytes at the base of the skull also were mentioned. This suggests that hydrocephalus, when it occurs in gargoyles, is due

to a skeletal abnormality which compresses the brain stem. A similar mechanism has been described by Grüneberg⁵³ in a mutation in mice which produces skeletal abnormalities and hydrocephalus by preventing drainage of cerebrospinal fluid from the fourth ventricle.

Among the well recognized anatomic changes are those found in the cornea in those cases in which corneal clouding existed. Although Kressler and Aegerter were able to demonstrate only artifacts in the cornea of their case,⁹ Berliner,¹⁷ Rochat,⁴⁴ and Zeeman⁴⁵ have described defects in Bowman's membrane, which contained vacuolated cells with granules in their cytoplasm. Berliner considered these as lipid granules. Rochat mentioned that the granules were soluble in ether and alcohol, not doubly refractile, and that they gave a pale yellow-brown stain with sudan III. In the case here reported the corneal changes were identical with those described by Berliner, Rochat, and Zeeman. No attempt was made to determine the chemical nature of the cytoplasmic granules. The ocular findings in gargoylism have been reviewed most recently by Cordes and Hogan.²⁸

Comparatively little attention has been paid thus far to the anatomic changes of the internal organs. Cardiac failure is often given as the apparent cause of death in gargoyles. The presence of hepatomegaly and splenomegaly has long been stressed as a part of the characteristic clinical picture. Splenectomy and a biopsy of the liver in a 2-year-old boy showing the classical picture of gargoylism (Ellis⁵⁴) revealed an enlarged spleen with hyperplasia of the pulp. The liver cells were described as "well filled with glycogen." A search for abnormal lipids was negative. A biopsy of the liver in the first case of Debré, Marie, and Thieffry³⁶ showed no histologic or histochemical abnormality.

Kressler and Aegerter⁹ were the first to stress the widespread visceral changes characterized by the presence of vacuolated cells in many organs, particularly the liver, spleen, lymph nodes, and myocardium. In the case presented here, the visceral changes were outstanding and so obvious that it seems strange that they should have escaped recognition for such a long time, unless there is a comparatively wide variability in their degree and extent, possibly related to age, or determined by modifying genetic factors. There are certain differences between the lesions described by Kressler and Aegerter and those in the present case. In the liver, involvement of the Kupffer cells was found in addition to extensive vacuolization of liver cells. On the other hand, the vacuolated cells in the portal areas mentioned by Kressler and Aegerter were not present.

The striking enlargement and vacuolation of the sinusoidal endo-

thelium in the spleen were not observed by Kressler and Aegerter⁹; they reported vacuoles in lymphocytes and reticulum cells not seen in the present case. Kny²⁴ found no microscopic changes in the spleen while the liver was described as showing early cirrhosis and vacuolation of the liver cells. The Kupffer cells, however, appeared free of changes.

Thus it seems that involvement of the reticulo-endothelial system is not a prominent or constant feature of gargoylism. Even in the present case, it is overshadowed by the striking alterations in the connective tissues of various organs. The extensive involvement of mesenchymal structures, such as the endocardium, the myocardial connective tissue, and the intima and media of large and medium-sized arteries, with an increase of collagen fibers apparently has not been observed by other writers. Kressler and Aegerter⁹ mentioned only infiltration of small vessels in the spleen, and although there was thickening of the mitral and tricuspid valves and streaking of the ascending aorta in their case, apparently the microscopic appearance did not correspond to what was seen in the case here reported. However, an increase of areolar connective tissue in the myocardium and vacuoles in the pericardium was mentioned by these authors.

Stoeckel,²¹ in his gross report, described chronic endocarditis with fibrous thickening of the valves and a glassy sclerosis of the aorta. Chronic endocarditis was mentioned also by Reilly¹⁶ and Wolff.²⁵ Njå³⁸ described thickening of the aortic and mitral valves, the latter showing knotty borders, as well as shortening and thickening of the chordae. The left ventricle was hypertrophied. There was no microscopic examination of these tissues. Yellow intimal plaques were observed in the aorta.

Although the gross appearance of the heart valves might have suggested chronic valvulitis, histologic investigation rules out this interpretation. The thickening of the valves is produced entirely by the accumulation of vacuolated connective tissue cells, together with an increase of collagen fibers and ground substance. The identity of the ballooned cells is not unequivocally established except for those which show the typical nucleus of the Anitschkow cell. Their localization and close association with newly formed collagen fibers support the view that for the most part they are fibroblasts rather than histiocytes. Attempts to demonstrate lipid substance in these cells have been unsuccessful. The same is true of the vacuolated cells in the spleen and liver, except that a small percentage of the vacuoles in the liver cells proved to be neutral fat.

Stains with mucicarmine, thionin, and basic fuchsin for mucin, car-

ried out on liver tissue fixed in absolute alcohol, as well as the toluidine blue stain on formalin-fixed tissue (mitral valve, aorta) also have been negative.

Reilly⁵⁵ found, in the cytoplasm of polymorphonuclear leukocytes in the peripheral blood, sternal marrow, and spleen, coarse granules which stained dark lilac with Giemsa's stain and which sometimes were eosinophilic. He observed these in 4 of 8 cases examined. Such granules were not observed in the present case.

Lesions of endocrine organs have been looked for by many writers, in an attempt to find an explanation for the pathogenesis of gargoylism. Reilly¹⁶ reported 3 cases of "an atypical familial endocrinopathy," which he later on included in the syndrome of gargoylism.⁵⁶ In one of these, autopsy had revealed changes in the thyroid and anterior lobe of the pituitary gland, as well as an enlarged thymus. Ashby and co-workers⁸ mentioned changes in the thyroid gland and a large thymus in their 2 cases, and enlargement and hyperplasia of chromophobe cells of the pituitary body in one. Kressler and Aegerter⁹ described the pituitary gland as enlarged and showing "infiltration" of chromophobe cells. In de Lange's case,²⁶ the pituitary gland showed degenerative changes and loss of chromophobe cells. These findings are not consistent, and no noteworthy changes have been found in the endocrine organs by other authors. In the present case the thyroid, pituitary body, thymus, adrenals, and ovaries were essentially normal.

The bone changes form a prominent part in the disease complex of gargoylism. Their rôle in the pathogenetic mechanism of this condition has been the subject of much thought and controversy. De Rudder⁵ felt that the dysostosis is not necessarily related to what he calls "Phosphatididiathese" (corneal changes, changes in the central nervous system, hepatosplenomegaly), but that each is transmitted through a recessive gene, and that the complex of Hurler's dysostosis results only when the two genes combine. In other words, he believed that the skeletal changes in gargoylism are unrelated to a general metabolic disorder.

It seems that only with a better knowledge of the genetic, anatomic, and chemical substrate may the rôle of the chondrodystrophic changes within this disease complex be elucidated.

The chondrodystrophic nature of the skeletal deformities has been apparent ever since the syndrome was first recognized, and the skeletal changes are the most constant clinical feature of this disease. In analogy with other known chondrodystrophies, this was thought to be a genetically determined anomaly. The first post-mortem studies of gargoylism did not concentrate on the histologic features of the bone

changes. At the most, the bone marrow was examined for lipid-storing cells which were never found. Kressler and Aegerter⁹ reported that microscopic examination of bone revealed "normal ossification and healthy bone growth." Among the first to call attention to the histologic bone changes were Washington,¹⁰ Kny,²⁴ and Schmidt.¹¹ Washington summarized the changes in the epiphyses as follows: Shortness of the zone of proliferating cartilage indicating slowness of cartilage growth, and formation of trabeculae disposed horizontally along the under surface of the epiphyseal cartilage as a result of the slowness of the process of endochondral ossification. The bony trabeculae lack calcified cartilage ground substance as a basis for the osteoblasts to build on. Washington mentioned storage in endosteal cells and osteoblasts, without elaborating on this finding.

Kny²⁴ also stressed that, while ossification was essentially normal, the growth process was markedly slow, particularly in the region of endochondral ossification. According to the classification of Kaufmann,⁵⁷ this is characteristic of chondrodystrophy of the hypoplastic type. Schmidt¹¹ found what he considered lipid storage in cartilage cells in the epiphyses, being able to stain some of the granules in their cytoplasm with the Smith-Dietrich method for lipoids. This storage was found mainly where normal proliferation of epiphyseal cartilage had failed to take place. He thought that the lipid storage interfered in some way with normal endochondral ossification. Thus he was the first to attempt an explanation of the chondrodystrophy as an integral part of the disease complex of gargoylism, rather than as an independent phenomenon as had been suggested previously (de Rudder⁵). Schmidt's findings as to lipid storage in cartilage cells could not be confirmed by Kny, nor could I do so.

As for the periosteum and perichondrium, Schmidt's findings¹¹ differ markedly from those in the present case. In the vertebra he described the periosteum as thickened, but no mention was made of the vacuolated cells observed by me. In the neck of the radius Schmidt found thickening of the cambium layer under the fibrous periosteum, but no abnormal cells. Since no membranous bones were examined by Schmidt, he did not elaborate on the pathogenesis of their deformity. Washington¹⁰ felt that the deformity of membranous bone in the skull could not be explained along with the chondrodystrophy, and he attributed it to a deficit in the "blastemal capsule" of the top of the skull. Sections through portions of the cranial vault in the present case showed vacuolated cells in the periosteum, and it is thought, therefore, that the disturbance of the function of the perichondrium and periosteum leads to the skeletal deformities. By what

mechanism this takes place is still open to question, especially since we do not know the nature of the severe changes in the bone-forming and other connective tissues.

An interesting histologic observation is that of the periosteal band [Perioststreifen] in a rib, which is a common finding in chondrodystrophy (Landauer⁵⁸).

The similarities and differences of gargoylism, chondrodystrophy, pléonostéose (Léri), Morquio's disease, and other hereditary diseases of the skeletal system have recently been discussed by Nöller⁵⁹ and Debré and co-workers.³⁶ A case of chondrodystrophy combined with mental retardation and bilateral corneal clouding was reported by Tröster,⁶⁰ who discussed its relation to gargoylism. Autopsy failed to reveal the typical changes of amaurotic idiocy in the brain and the characteristic visceral involvement of gargoylism. The nature of the corneal clouding was not investigated.

It appears that although the syndrome of gargoylism generally is very uniform, there are cases which do not present the fully developed clinical picture. These are so-called intermediate forms, or formes frustes, which share features both with gargoylism and with other known diseases having apparently a similar anatomic and genetic substrate. For example, the group of cases reported by Jervis,⁴⁷ which have been discussed above, belongs in this intermediate class. Cases with normal or almost normal mentality have been reported by Hunter,¹ Reilly,¹⁶ Lahdensuu,³⁰ Nonne,⁶¹ Liebenam,⁶² Cockayne,⁶³ and others. One has to assume that the central nervous system in these cases lacked the severe changes described in some post-mortem studies. Nevertheless, the other stigmata of gargoylism were present in the majority of these patients, except for the absence of corneal clouding in some. Absence of corneal changes is mentioned also in the case reports of Ross, Hawke, and Brown,²² Lurie and Levy,³⁴ Debré and co-workers,³⁶ Njå,³⁸ and de Lange and Woltring.⁶⁴ In all reported instances of gargoylism with normal corneae the patients were males, and usually the disease was present in siblings or, as in Njå's report, in 5 male members of one family. Njå's postulation of gargoylism of a special sex-linked type with normal corneae is of interest in this connection.

De Lange and Woltring⁶⁴ were hesitant to consider their 2 cases as examples of gargoylism because of the absence of corneal clouding, kyphosis, and widening of the sella; also, because mental retardation was not very marked. They designated them as "typus E" after the initial of the patient's family name. The photographs of the patients,

showing the typical facies and dwarfism, the presence of hepatosplenomegaly and skeletal deformities, seem to justify the inclusion of these cases in the group of gargoylism. In the meantime, one of the 2 brothers has been autopsied at the age of 19 years⁴⁶ (See Table II). The lack of cerebral changes is difficult to explain in view of manifest mental retardation at the time of the patient's death. It appears that in the case of typus E, lesions were most striking in mesenchymal structures such as the perichondral tissues of the larynx, trachea, bronchi, and elbow joint, and in the heart valves and aorta. What is described as a "chondroid" appearance of the heart valves and of the perichondrium may very well be identical with the changes described in the present case of gargoylism. It is not quite clear what the writers meant by mucoid degeneration of the connective tissue; possibly it corresponds to the peculiar swelling of the ground substance described in the heart valves of the case here reported. It is possible, but not proved, that in the case of typus E, vacuolation of the liver cells was due entirely to deposition of glycogen. Mild cirrhosis of the liver has been observed by others in typical cases of gargoylism. The lack of storage in the spleen apparently does not speak against gargoylism. Discrepancy in the skeletal findings may be accounted for by the difference in age at the time of death of the case of typus E and of this case. It appears that typus E constitutes a most interesting link between the formerly described classical cases of gargoylism and the case reported here. Several genetic mechanisms may account for the variability in the expression of gargoylism and other hereditary disorders. One of these is the existence of genetic or environmental modifiers similar to those which have been demonstrated in genetic studies in laboratory animals, for instance, taillessness in the rat.⁶⁵ Another possibility is that the severity and the time of onset vary with the mode of inheritance (dominant, recessive, or sex-linked) as has been demonstrated for retinitis pigmentosa⁶⁶ and peroneal atrophy.⁶⁷

It has been suggested that histologically or biochemically detectable storage of substances may be due to a genetically determined enzyme deficiency.⁶⁸⁻⁷⁰ In some of the human storage diseases the accumulated substance has been chemically identified. It is glycogen in von Gierke's disease, sphingomyelin in Niemann-Pick's disease, kerosin in Gaucher's disease, neuramic acid in Tay-Sach's disease. In gargoylism chemical identification has not been possible until now.* However, the morphologic similarity of the histologic changes with those in other storage diseases suggests that here too the basic abnormality

* Chemical investigation of organs in the case here reported is being carried out.

is a hereditary disturbance of metabolism. Thannhauser and Schmidt⁷¹ have stressed that the stored substance accumulates not only in the reticulo-endothelial system, but in many tissue cells as well. They therefore assumed that intracellular metabolism is disturbed in these diseases. The involvement of a variety of tissue cells, and particularly of the connective tissues, in gargoylism seems worth mentioning in this connection.

It seems premature to conclude that gargoylism is a lipid storage disease, as long as the stored substance is unknown. The presence of cells with a foamy cytoplasm is not necessarily the result of storage of fat or fat-like substances. It occurs in glycogen storage disease, and has been produced in experimental animals after the administration of nonlipid substances of high molecular weight.⁷²

SUMMARY

A case of gargoylism in a 3-year-old girl, with gross and microscopic post-mortem examination, provided an opportunity for comparing the reported findings with those in the present example.

The previously described lesions in the central nervous system, eyes, skeleton, and visceral organs are for the most part confirmed. Emphasis is placed on striking alterations in the connective tissues of the viscera, cardiovascular system, and skeleton, which hitherto had not been observed. These are characterized by the presence of large vacuolated cells, probably fibroblasts for the most part, in association with a proliferation of collagenous fibers and sometimes with an increase of ground substance.

These alterations have a rôle in producing some of the characteristic clinical manifestations of gargoylism (hydrocephalus, skeletal deformities, cardiac symptoms).

The nature of gargoylism must be considered in its relation to the known diseases of lipid metabolism (amaurotic idiocy, Niemann-Pick's disease, Gaucher's disease). The inclusion of gargoylism in the group of storage diseases is suggested because of the evidence of storage in many cells of the body, and because of the similarity of the changes in the central nervous system with those in amaurotic idiocy; but the chemical nature of the stored substance thus far has not been identified. It appears that there is a widespread disturbance of intracellular metabolism resulting in the accumulation of an abnormal substance in many cells of the body. However, the question whether gargoylism is a form of lipidosis must remain open.

I wish to express my sincere appreciation to Dr. P. Klemperer for his invaluable guidance in preparing this paper.

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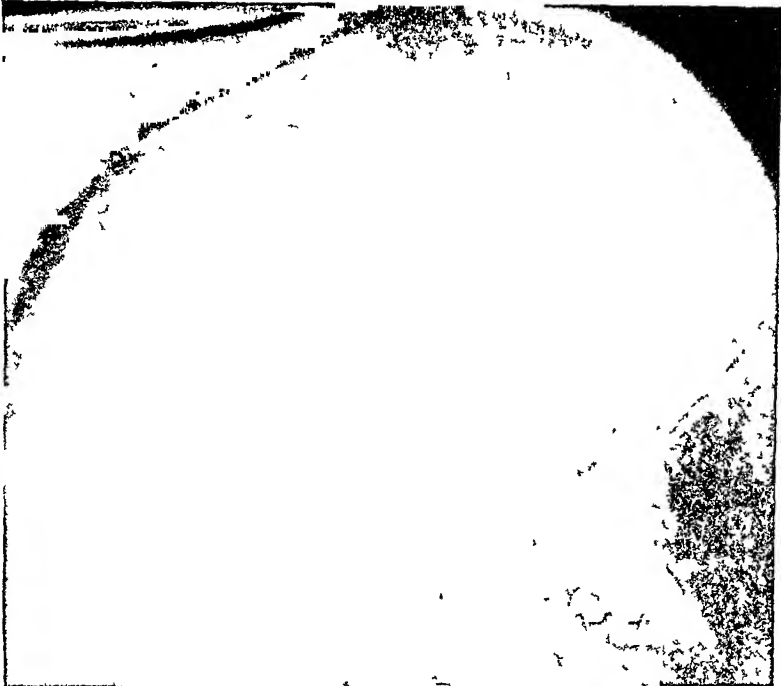
[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 138

- FIG. 1. Typical gargyle facies of patient (post-mortem photograph).
- FIG. 2. Roentgenogram of skull showing the marked scaphocephalic deformity.
- FIG. 3. Heart. Hypertrophy of the left ventricle. Nodular, fleshy thickening of the mitral valve. Shortening and thickening of the chordae tendineae.
- FIG. 4. Heart. Concentric hypertrophy of the left ventricle. Thickening of the parietal endocardium in the aortic outflow tract. Thickening of the aortic valve leaflets. Intimal plaques in the ascending aorta.
- FIG. 5. Lumbar spine. Sagittal section through vertebral bodies showing deformity with "beaking" of the antero-inferior portions. (The anterior border of the vertebrae corresponds to the upper border of the figure.)

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Strauss

Gargoylism

PLATE 139

- FIG. 6. Myocardium. Patch of interstitial fibrosis containing vacuolated cells. Hematoxylin and eosin stain. $\times 475$.
- FIG. 7. Mitral valve and left atrium. There is striking thickening of the atrial endocardium and the mitral valve. Hematoxylin and eosin stain. $\times 10$.
- FIG. 8. Mitral valve. The valve is made up of large vacuolated cells associated with thick bands of collagen fibers and a homogeneous ground substance. Some of the cells are arranged in rows. Hematoxylin and eosin stain. $\times 475$.
- FIG. 9. Aorta containing a large intimal plaque. Weigert's elastica and van Gieson's stain. $\times 10$.

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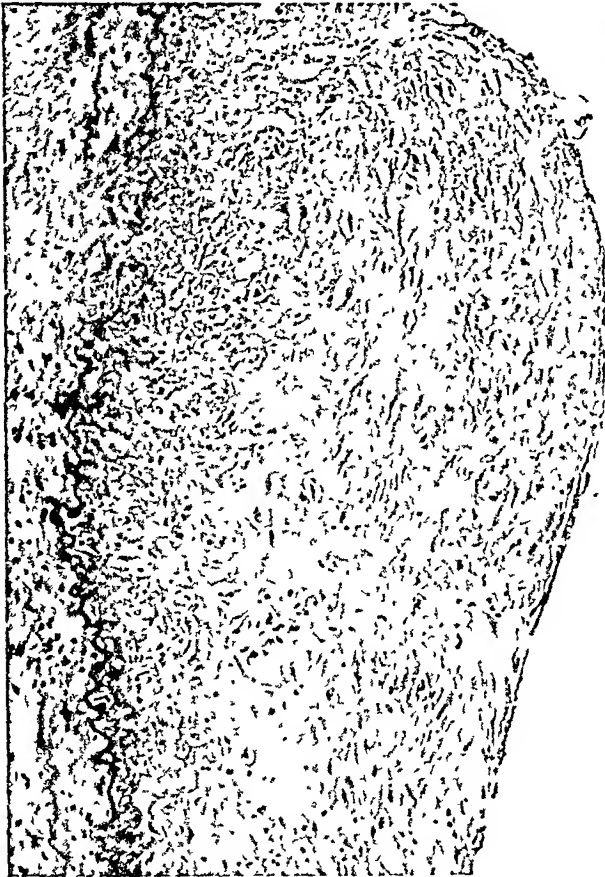


Gargoylism

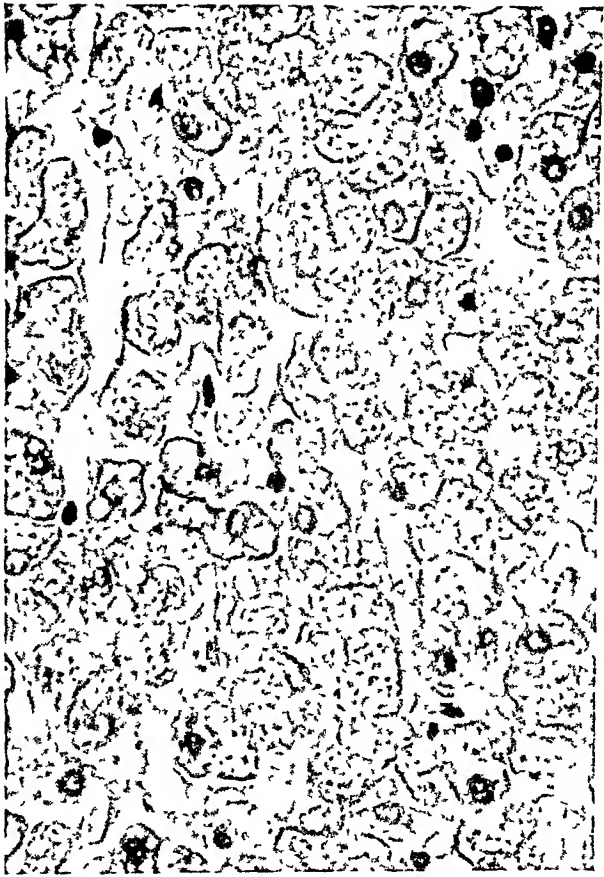
PLATE 140

- FIG. 10. Intimal plaque in a branch of the superior mesenteric artery. Hematoxylin and eosin stain. $\times 75$.
- FIG. 11. Liver. The liver cells are enlarged and have a honeycombed appearance due to diffuse vacuolization. There is no fibrosis. Mallory's aniline blue-orange G stain. $\times 380$.
- FIG. 12. Spleen. The endothelial cells lining the sinusoids are enlarged and vacuolated. The sinusoids are empty. The follicles are depleted of lymphocytes. There is no infiltration of the intersinusoidal reticulum. Mallory's aniline blue-orange G stain. $\times 300$.
- FIG. 13. Cornea. There are large vacuolated cells interposed between the corneal epithelium and the substantia propria, replacing Bowman's membrane. Hematoxylin and eosin stain. $\times 475$.

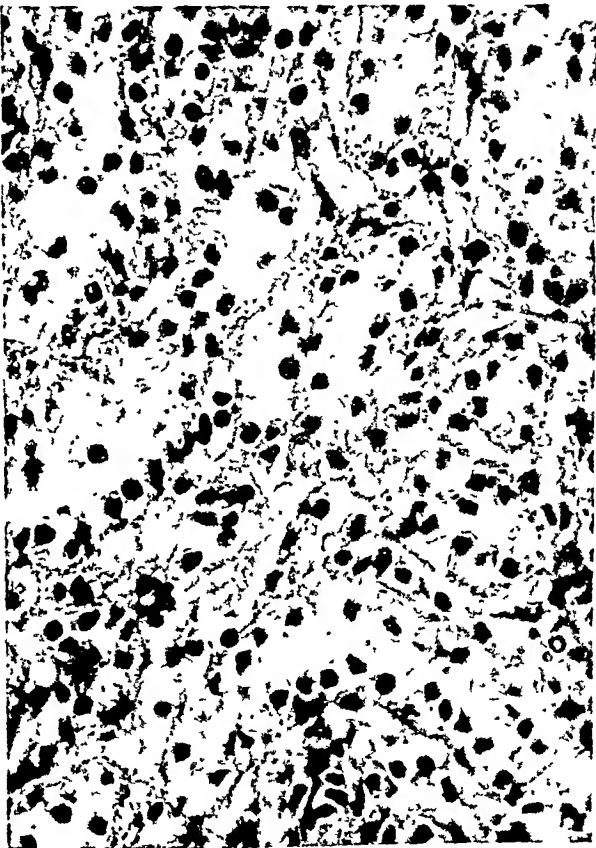
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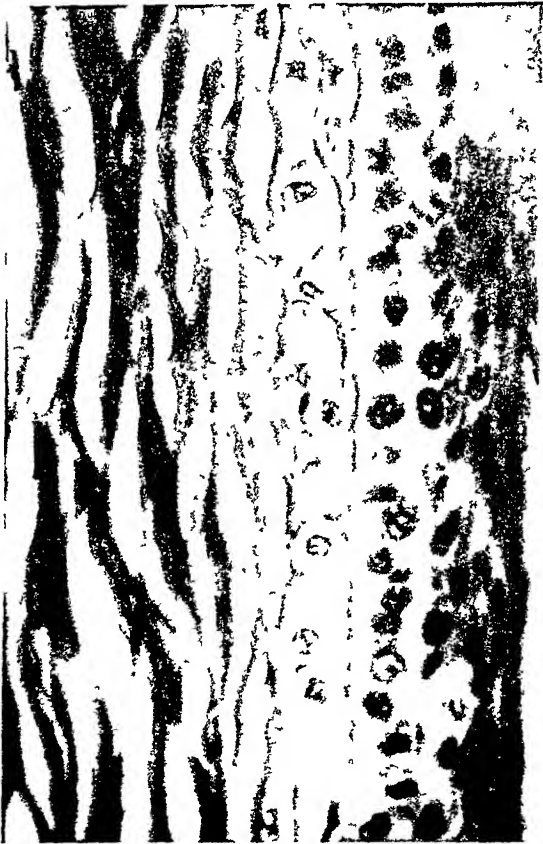
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Strauss

Gargoylism

PLATE 141

- FIG. 14. Rib. The center of the epiphyseal zone shows replacement of the proliferating cartilage by a vascular connective tissue containing vacuolated cells. A transverse plate of bone is laid down along the epiphyseal line. Hematoxylin and eosin stain. $\times 200$.
- FIG. 15. Rib. Cortex with adjacent periosteum which is thickened and diffusely infiltrated with vacuolated, spindle-shaped cells. The vacuolated cells penetrate into lacunae in the cortex. Hematoxylin and eosin stain. $\times 300$.
- FIG. 16. Vertebra. The epiphyseal zone shows the cartilage in the resting stage. There is no evidence of proliferation or arrangement of cartilage cells in columns. Ossification is of the perichondral type. Hematoxylin and eosin stain. $\times 200$.
- FIG. 17. Vertebra. Epiphyseal zone shows fair proliferating activity of the cartilage and endochondral type of ossification (for comparison with Fig. 16). Hematoxylin and eosin stain. $\times 200$.

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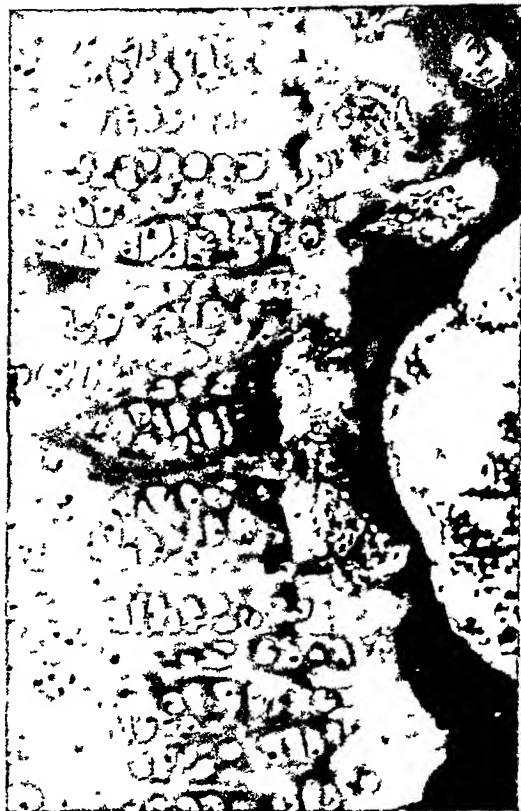
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Strauss

Gargoylism

STUDIES ON PERIARTERITIS NODOSA

III. THE DIFFERENTIATION BETWEEN THE VASCULAR LESIONS OF PERIARTERITIS NODOSA AND OF HYPERSENSITIVITY *

PEARL M. ZEEK, M.D., CARL C. SMITH, Ph.D.,† and J. C. WEETER, M.D.

(From the Department of Pathology, Cincinnati General Hospital, Cincinnati, Ohio)

Recent medical literature contains frequent reference to lesions which resemble periarteritis nodosa and which occur in certain cases of hypersensitivity to various substances, especially to the sulfonamides. The occasional association of a necrotizing type of panarteritis with hypersensitivity has been recognized for many years. The identification of these lesions as those of periarteritis nodosa has not yet been proved.

Recent studies in this laboratory on necrotizing panarteritis have revealed cases of two types which are recognizable both pathologically and clinically. One type is associated with hypersensitivity. For convenience, in this report the lesions of this condition will be called hypersensitivity angiitis, both arteries and veins usually being involved. For the other type the term periarteritis nodosa will be retained. The term necrotizing panarteritis will be used generically to include not only the lesions of these two conditions, but also any other vascular lesions which are characterized at some stage in their development by necrosis and inflammation involving all three coats of vessel walls.

In this report we will describe how periarteritis nodosa and hypersensitivity angiitis differ morphologically. The clinical manifestations will be described in a subsequent report. One case of each type has already been reported, one by Williams and Zeek¹ and the other by Thompson and Zeek.²

METHODS

Tissues from experimental animals included those from 262 rats used in the 7 experiments of our first two studies on periarteritis nodosa^{3,4} and 20 rats used in experiment 8 which will be described in this report.

Human tissues were from 31 cases, with autopsies, as follows: 29 cases of necrotizing panarteritis encountered in the Department of Pathology of the Cincinnati General Hospital over a period of 10 years, and one case each from the Children's Hospital and the Good

* Received for publication, August 6, 1947.

† Now at Institute of Medical Research, Christ Hospital, Cincinnati, Ohio.

Samaritan Hospital of Cincinnati, which we have had the opportunity to study through the courtesy of the pathologists of these hospitals.

In two previous reports on periarteritis nodosa,^{3,4} we described the occurrence, and later the production, of periarteritis nodosa in rats which were made hypertensive by the silk-perinephritis method. In the 262 rats whose organs and tissues were examined both grossly and microscopically the occurrence of periarteritis nodosa was strictly limited to those animals which had silk placed around both kidneys, or had silk around one kidney and the other one removed. Sixty-two of the rats thus treated presented typical lesions of periarteritis nodosa at autopsy. The lesions were not found in any of 43 controls which were not operated upon, nor in various series of animals which had silk placed in the abdomen elsewhere than around the kidneys, nor in any of 21 rats in which only one kidney was operated upon. In some cases an interval of 1 week or 10 days elapsed between the operations on the two kidneys, but this interval played no rôle in the production of periarteritis nodosa since lesions occurred with similar frequency in those rats which had both kidneys operated upon the same day.

The statistical significance of these figures justifies the conclusion that in this series of rats periarteritis nodosa did not begin to develop before the day on which the second kidney was operated upon. For convenience this day is designated as the K-2 date, and is considered the earliest date of initiation of lesions. The length of life after that date in the 62 rats with periarteritis nodosa varied from 7 days to 52 weeks.

Although K-2 date is considered the earliest date on which the lesions could have begun, there is no reason to believe that lesions may not have begun after that date. However, some of the rats died within 7 to 14 days after operation and had well developed, typical, necrotizing exudative lesions in vessels (Fig. 8). Therefore, it is evident that some of the lesions must have begun very soon after K-2 date.

On the other hand, sections from animals living 10 to 52 weeks after K-2 date presented not only scarring and distortion of vessel walls (Fig. 11) but also, in many cases, fresh, active lesions similar to those found in animals living less than 2 weeks. In many cases lesions of various ages were found in different vessels of the same organ. Thus it is evident that many of the animals which lived a long time after operation had experienced multiple episodes of initiation of lesions, and whatever caused the lesions did not produce its effect once only, but recurred in bouts throughout the rest of the animal's life even for as long as 52 weeks after onset of the first lesions.

To be classified as "positive" in our previous studies, a case had to present at least one typical, full-blown lesion of periarteritis nodosa (Fig. 8) in which there was fibrinoid necrosis of the wall of a small or medium-sized artery, with an inflammatory cellular reaction of pleomorphic type involving all three coats. One or more such lesions were found in each of the 62 rats. In 19 other rats arterial lesions were found which did not fulfill these criteria in all respects. These lesions were listed as "questionable." No such lesions were found in any of the 43 controls, but similar lesions occurred in many of the 62 rats which presented also typical lesions of periarteritis nodosa. Some of the 19 rats which had "questionable" lesions only, were those which had died or were killed in less than 7 days after K-2 date. It seemed reasonable to consider such lesions as early stages of periarteritis nodosa. Experiment 8 was performed to furnish more material for the study of such early lesions.

Experiment 8. Twenty young, healthy, white male rats, weighing between 315 and 435 gm. each, were obtained from a group of several hundred similar rats, the rest of which were used for other experiments and none of which showed any evidence of periarteritis nodosa grossly or microscopically. These 20 rats were operated upon and housed in a different building from that used in our previous experiments. On December 12 all 20 rats were subjected to a silk-perinephritis operation (silk was placed around one kidney and the other kidney was removed). As the operations proceeded, alternate kidneys were wrapped or removed respectively. Nembutal anesthesia was used. Ether had been used in the previous experiments. One rat died during the operation, another 2 hours later, and 3 died within the next 2 days. The other 15 were sacrificed with chloroform anesthesia on various postoperative days as follows: 3 on the 3rd day, 4 on the 7th day, 2 on the 11th day, 3 on the 14th day, and 3 on the 24th day. Autopsies were performed and sections from the various organs and tissues were taken for microscopic study. All 12 rats which lived to the 7th postoperative day or longer presented one or more full-blown, typical lesions of periarteritis nodosa similar to that shown in Figure 8. Those dying on the first and second days after operation presented no recognized vascular changes other than perivascular edema. In those animals of this series and of our previous series which died on the 3rd to 6th day, departures from normal were found which undoubtedly presented the early stages of periarteritis nodosa. All gradations in the process have been seen in the numerous sections studied, and similar changes were never seen in any of the controls. Similar changes were frequently found in tissues from rats of our previous series which also presented both healed and

fresh lesions and had died during a time when new lesions were being initiated.

Thus, our experiments on periarteritis nodosa have afforded us the opportunity of observing the very early lesions of this condition and of tracing their development for as long as 52 weeks after the earliest date of initiation of lesions. The illustrations show various stages which will now be described.

PERIARTERITIS NODOSA IN THE RAT

First Stage. The first departure from normal in the vessels of the rats which were subjected to the silk-perinephritis operation appeared on the 2nd and 3rd days after K-2 date but were seen also in animals, dying at longer intervals, which presented also typical full-blown lesions. These early changes consisted of fragmentation, degeneration, and focal edema of adventitial collagen at the bifurcation of arteries of muscular type near the site of their entrance into viscera. Occasionally, a vessel seemed to be surrounded by a ring of edematous stroma, but more often the change was focal and eccentric. The departures from normal in this stage were not striking and had to be looked for carefully in the right places or they would have been missed.

Second Stage. Proliferation of fibroblasts and histiocytes occurring in the focal areas of adventitial degeneration characterized the second stage, and was found in some rats on the 3rd day after K-2 date. The lesion occasionally appeared as a ring of fibroblasts surrounding the vessel (Fig. 1) but more frequently was seen as an eccentric collection of somewhat hyperchromatic cells in the adventitia (Fig. 2), often near a bifurcation, and often involving only the adventitia of the adjacent aspects of the two distal vessels. The lesion appeared to spread distally, proximally, and circumferentially from the point of origin. Frequently the long axes of the proliferating fibroblasts lay at right angles to the axes of the smooth muscle fibers of the outer media, producing a radiation effect. Often the demarcation between the media and adventitia was obliterated and the media seemed to fade out into the radiating zone of fibroblasts and histiocytes in the adventitia. Occasionally, among the first cells to infiltrate the adventitia were a few large, oval cells loaded with conspicuous round granules which had the staining qualities of mast cells when Giemsa's and acid fuchsin stains were used. When the vessel lay in adipose tissue at the hilum of a viscus there was often marked histiocytic proliferation in the surrounding fat as though there were dispersion of some injurious substance outward from the vessel wall.

Third Stage. Necrosis and inflammatory exudation characterized the third stage and produced the typical full-blown lesions which have long been known as periarteritis nodosa (Fig. 8). The earliest appearance of such lesions after K-2 date was in 5 rats which died or were killed on the 7th day, and in 6 rats on the 11th day. Necrosis apparently began in the outer media or inner adventitia, was never seen to be circumferential before exudation occurred, but often appeared as a streak of necrosis through the thickness of the media at a site near a bifurcation (Fig. 4). The necrosis appeared fibrinoid with hematoxylin and eosin, but with trichrome and Mallory's aniline blue stains the "fibrinoid" material stained more like hemoglobin than like fibrin.

Inflammatory exudation followed rapidly; the cellular exudate was always pleomorphic in type and often included varying numbers of eosinophils. Foreign body giant cells were never found in these vascular lesions. Fibroblasts and histiocytes also increased in numbers in this stage, and new capillaries appeared in the adventitia giving to the perivascular nodules the appearance of granulation tissue. In some vessels necrosis and exudation rapidly involved the whole circumference and extended both distally and proximally from the points of bifurcation into the distal and proximal vessels. Often thrombosis and occasionally aneurysmal dilatation occurred at this stage. In some vessels the lesion progressed into the healing stage without involving more than a small sector of the vessel wall.

Fourth Stage. In the fourth stage, healing occurred by the formation of granulation tissue (Fig. 10). Granulating lesions were common in tissues of rats as early as 2 and 3 weeks after K-2 date.

Fifth Stage. The scars thus formed were so unusual in structure as to be possibly pathognomonic of healed, or fifth stage, periarteritis nodosa. They were characterized by a mass of vascularized fibrous tissue completely replacing a sector of a vessel wall which, with serial sectioning, was shown to be near a bifurcation of an artery of the muscular type, often in the hilar region of a viscus. In cases with extensive involvement, arteries distant from the hilar regions of certain viscera also were affected. In some vessels the scarring was completely circumferential in a given segment of a vessel (Fig. 11). In a few vessels the lesions presented scarring of the adventitia and a marked proliferation in the intima, sometimes with a canalizing, organizing thrombus, but with a normal-appearing media. Serial sections showed that such lesions were continuous with scars which bisected the media in a limited area only, while the adventitial and intimal reaction had extended distally and proximally from the initial point of

injury. To interpret lesions of periarteritis nodosa properly, it is essential to think of them in terms of four dimensions—three of space and one of time.

Scarred lesions of periarteritis nodosa occasionally became calcified. The calcium was deposited haphazardly in the hyalinized scar tissue, without any preceding atheroma, and with no predilection for any particular part of the vessel wall. Thus the lesion can be differentiated readily from other common forms of arteriosclerosis (Fig. 11).

Several observations concerning the gross distribution of the lesions seem noteworthy. No lesions of periarteritis nodosa were found in the arteries of the pulmonary circulation although they were looked for carefully in numerous sections from the different parts of the lungs. Many of the rats, including controls, had peribronchial lymphoid hyperplasia, and sometimes blood vessels as well as bronchi were surrounded by nodules of lymphoid tissue. This lesion should never be confused with periarteritis nodosa. When the heart and lungs were removed from the body *en masse*, hilar sections of the lungs often included other structures such as portions of the pericardium, heart, and esophagus. Vessels of these structures often presented lesions of periarteritis nodosa. In one case of widespread advanced lesions throughout the body, except in the lung, one small artery in the wall of a primary bronchus presented a typical lesion. The bronchial arteries usually arise from the general systemic circulation and should not be confused with the pulmonary system.

In over 90 per cent of our male rats with periarteritis nodosa in the abdomen or chest, lesions were found also in the primary branches of the spermatic arteries, near the site of their entrance into the testes and epididymides. This presents a site for biopsy in experimental animals (Fig. 6). Lesions were found far more frequently in this location than in sections of striated muscle from the same rats.

In the early stages of periarteritis nodosa, the intrinsic vessels of the viscera usually were normal in appearance. In the late stages, small arteries in the portal areas of the liver, the arteries between the cortex and the pyramids in the kidneys, and most of the arteries in the pancreas were involved. In the mesenteric vessels the lesion began in the arteries nearest the sites of the mesenteric attachments to the wall of the gut, and extended distally into the wall of the intestines from these points, and proximally to involve the larger mesenteric arteries (Fig. 15).

In the heart the first vessels to show departures from normal were

those at the base near the origin of the coronary arteries, and those in the subepicardial portion of the myocardium of the ventricles. For some unknown reason the arteries of the right ventricle often were involved before those of the left side. In late stages there often was widespread involvement of the coronary arteries, with extensive infiltration of the myocardium and endocardium with histiocytes, and less frequently with other inflammatory cells. Small infarcts were common. No lesions similar to Aschoff bodies were found in any of these rats.

Periarteritis nodosa of the splenic vessels was almost invariably limited to the larger arteries at the hilum. In only 2 cases did it extend into the spleen far enough to involve several arterioles. The follicular arterioles in 60 rats, with well developed lesions elsewhere, appeared normal, except for moderate arteriolar sclerosis.

Veins were involved occasionally in cases with extensive lesions in arteries, but only by direct extension of the inflammation from an adjacent artery (Fig. 6). Fibrinoid necrosis was not seen in veins in cases of periarteritis nodosa. Moderate sclerosis of arterioles of the type seen in hypertension was common in these rats. It is easily differentiated from periarteritis nodosa by the absence of cellular reaction and fibrinoid necrosis.

Many of the rats developed infarcts and fibrosis of various viscera as the result of vascular occlusion by periarteritis nodosa. Some died from these lesions while others with less extensive lesions lived until sacrificed many months after operation. Those which died usually showed new lesions as well as old healed ones.

Special stains have not given much aid in determining the cause of periarteritis nodosa. Elastic tissue stains revealed early loss of the external elastic lamella at the site of lesions. Foot's reticulum stain showed marked proliferation of reticular tissue in the adventitia beginning in the second stage and becoming more marked as new capillaries were formed in the third stage. Bacterial stains revealed no organisms related to the vascular lesions, although infected pus pockets often were found in the perinephritic hulls formed by the silk. As stated in a previous report, a variety of organisms were isolated, but similar ones also were found in the pus pockets related to silk placed around the spleens in 18 rats which did not develop any lesions of periarteritis nodosa. Therefore, the only rôle of organisms in the production of periarteritis nodosa in these series of rats seemed to be as follows: In rats with both kidneys operated upon, when the silk was contaminated by any of a variety of organisms, the perirenal tissue reaction was greater, the hull formed was heavier, the renal ischemia

was increased, the blood pressure was apt to be of a higher, spiking type, and the animal was more likely to present periarteritis nodosa at autopsy.

PERIARTERITIS NODOSA IN MAN

The autopsy reports and the microscopic sections of 31 human cases of necrotizing panarteritis were reviewed and the findings compared with those in the rat. Special attention was given to vascular lesions which were in early, pre-exudative stages of development at the time of the patient's death. In 15 cases, morphologic changes were found which furnished convincing evidence that the vascular lesions were of the same type as those in the rat (Figs. 3, 5, 7, 9, 12, 13, and 14). The various phases of the early stages of the lesions were found; their relation to the bifurcation of vessels was evident in serial sections; the distribution of the lesions, including their absence in the pulmonary circulation and in the arterioles of the spleen, was strikingly similar; and 12 cases presented, in addition to full-blown active lesions, healing stages of the characteristically scarred lesions of periarteritis nodosa. Three cases were acute and fulminating, the patients dying within a few weeks after the onset with involvement of multiple systems. The lesions in these cases were comparable to those which were found in rats which died about 2 weeks after K-2 date.

HYPERSENSITIVITY ANGIITIS IN MAN

Seven of the other 16 cases in man presented vascular lesions which were different from those just described, but which were strikingly similar to one another. All of these cases had presented clinical evidence of hypersensitivity during the last month of life. In 6 cases the reaction followed the administration of some therapeutic agent, usually one of the sulfonamides. The lesions in these cases were widespread in the viscera, including the lung. The seventh case was that of a young woman with a long history of severe asthma who died during an attack. The vascular lesions were of the type to be described, similar to those of the other 6 cases, but limited to the vessels of the lung.

We have called the lesions in these 7 cases hypersensitivity angiitis. The fully developed lesions consisted of fibrinoid necrosis of the walls of small vessels with pleomorphic cellular exudation within and around the vessel walls. This process was very similar to that seen in the third stage of periarteritis nodosa except that fibroblasts and new capillaries were absent or very inconspicuous and thus the formation of granulation tissue in and around the vessel wall was not seen (Fig. 16). However, such lesions could easily be confused with those of periarteritis nodosa. To differentiate between them it was important

to observe the distribution of the lesions and the structure of the early lesions.

In the 6 cases in which the lesions were widespread, they were found in the small intrinsic venules, arterioles, and small arteries of the viscera, including the lung (Fig. 18), while the larger arteries of the muscular type were rarely involved. The lesions showed no predilection for sites of bifurcation although these were involved occasionally. Lesions were common in the walls of portal veins within the liver even when adjacent arteries appeared normal (Fig. 20). Fibrinoid necrosis occurred in veins as well as in arteries. The follicular arterioles in the spleen were extensively involved (Fig. 17), while the arteries at the hilum usually appeared normal. Frequently there was extensive inflammation in the splenic trabeculae. This has been described as trabeculitis by More, McMillan, and Duff⁵ in cases of sulfonamide allergy. In our cases the inflammation often was limited to the intima of veins in the trabeculae, and occasionally there were small foci of necrosis in the vascular collagen (Fig. 19). Therefore, we consider this venous lesion as only another manifestation of the generalized vascular disease of hypersensitivity. Cellular reaction alone in this location is not pathognomonic of hypersensitivity. It is common in many toxic and infectious conditions without other manifestations of allergy.

The vascular lesions in the viscera were usually accompanied by edema of the interstitial tissue and small foci of necrosis. Occasionally, the interstitial tissue was infiltrated with inflammatory cells of various types, including eosinophils and eosinophilic histiocytes. All of these cases also presented an unusual type of necrotizing glomerulonephritis which was not seen in any of our cases of periarteritis nodosa, although 3 of the 15 presented the usual type of diffuse glomerulonephritis. In the cases of hypersensitivity the glomerular lesions (Fig. 21) were characterized by exudation and necrosis rather than by the proliferation of epithelium and endothelium so common in the usual glomerulonephritis.

A very important criterion in the differential diagnosis of these two types of vascular lesions concerns the structure of pre-exudative lesions. In patients dying of periarteritis nodosa there are usually lesions in all stages of development and it is easy to find vessels which show early lesions. Even in our 3 acute fulminating cases the lesions were not all of the same age, although none had progressed much beyond the third stage. In our 6 cases of hypersensitivity all of the lesions appeared to be much more nearly of the same age and it was difficult to find pre-exudative lesions in patients who died within a few days after the onset of hypersensitivity. However, careful evaluation of other

factors already discussed aided in making the diagnosis in such cases. In patients who lived for several weeks, the vessels which were not normal, but which did not present full-blown lesions, showed fibrinoid necrosis of the intima and inner media, often completely surrounding the lumen. In some of the lesions the entire wall of small vessels presented fibrinoid necrosis without any cellular reaction, but this was unusual. Inflammatory reaction evidently followed necrosis promptly.

No evidence of healing was seen in any of our cases of hypersensitivity. Although certain morphologic manifestations of the hypersensitive state (such as acute nonsuppurative interstitial nephritis, hives, and interstitial edema of viscera) regress, leaving no landmarks, it seems unlikely that the necrotizing lesions of arterioles, such as commonly occurred in the splenic follicles in these cases, could ever heal completely, leaving no stigmata. One wonders whether the periarteriolar follicular fibrosis and the chronic degenerative changes in vascular collagen of disseminated lupus erythematosus could possibly be the manifestation of chronic or repeated hypersensitivity. These and certain other lesions of the condition would fulfill satisfactorily our expectations of the morphology of chronic hypersensitivity angiitis.

OTHER TYPES OF NECROTIZING PANARTERITIS IN MAN

So far, two morphologic types of necrotizing panarteritis have been presented, which include 22 of our 31 cases. Probably there are other types to be recognized in the future. The remaining 9 cases presented lesions which did not satisfactorily fulfill the criteria established for the two categories discussed. In some of these the variations in the lesions of periarteritis nodosa were caused by the presence of other disease which also affected vessels, such as syphilis, brucellosis, and rheumatic fever. For the sake of clarity these cases were omitted from the series discussed. A case of fulminating tuberculous meningitis presented necrotizing panarteritis of the meningeal arterioles and venules very similar to hypersensitivity angiitis, but with the added component of tuberculous reaction with giant cells. That these lesions reflected an allergic state is in agreement with our present concept of this form of tuberculous disease.

Two of the 9 unclassified cases presented lesions which may represent a combination of hypersensitivity angiitis and periarteritis nodosa. A discussion of this subject would be incomplete without mention of the fact that allergic reactions occasionally occurred in the terminal stages of cases of long-standing periarteritis nodosa in which careful clinical studies over periods of several years revealed no other manifestations of allergy. Four of the 15 cases of periarteritis nodosa, for

no apparent reason, suddenly developed urticaria and eosinophilia during the last few weeks of life, with no previous recognized manifestation of allergy. Two of these had been diagnosed periarteritis nodosa by biopsy before the appearance of urticaria. Another case had a long history of disease of multiple systems with fever, tachycardia, hypertension, and polyneuritis. Three of the 4 cases presented at autopsy many older lesions which certainly long antedated the only recognized clinical manifestation of allergy. None of these 4 cases presented any lesions of the type classified above as hypersensitivity angiitis. The basis for individual variations in the manifestations of allergy still remains a mystery.

However, considering these 4 cases, it was not surprising that in 2 other cases (listed in this report as unclassified), which presented terminal allergic manifestations clinically, lesions of both types were found. In these cases also, no clue to the cause of the hypersensitivity was found in the clinical data. An interesting conjecture is whether the patients could have become sensitized to some abnormal protein substance produced by the extensive necrosis of their vascular tissues. Such combinations of lesions, as well as the morphologic similarity between their full-blown stages, account for much of the confusion between the two conditions.

CERTAIN COMMENTS ON THE LITERATURE OF NECROTIZING PANARTERITIS

Since the entrance of the sulfonamides into the field of therapeutics many case reports have been added to the already voluminous literature on necrotizing panarteritis. Many authors have limited their descriptions of lesions to the statement that their cases presented the "typical lesions of periarteritis nodosa." A review of the more detailed descriptions in the available medical literature of over 200 cases which were called periarteritis nodosa or one of its common synonyms such as polyarteritis nodosa or necrotizing panarteritis, has failed to reveal any one morphologic change common to all cases, except that at some time during the evolution of the lesions some pathologic change involved the entire thickness of the vessel walls. This change usually was described as "fibrinoid" necrosis, which is still an ill defined entity both chemically and pathogenetically, if not morphologically.

According to the literature* the degenerative process may begin in the intima and spread outward (Graf,⁶ Weir⁷), or in the adventitia

* For the sake of brevity, only a few representative references are given for each of the following opinions. More complete bibliographies may be found in several of the articles to which reference is made.

and spread inward (Kussmaul and Maier,⁸ Ophüls,⁹ Jacobsen¹⁰), or in the media and spread both ways (Arkin,¹¹ Mönckeberg,¹² Lichtman, Stickney, and Kernohan¹³). The exudate may be composed chiefly of eosinophils (Ophüls⁹), or be pleomorphic (Arkin,¹¹ Grant¹⁴), or may even contain giant cells (Weinberg,¹⁵ Cabot case 31241¹⁶). The lesions may be predominantly in arterioles (Miller and Nelson¹⁷), or may involve small and medium-sized arteries of the muscular type (Kussmaul and Maier,⁸ Haining and Kimball¹⁸). There may be either extensive or rare involvement of veins, or of the pulmonary arteries. Lamb¹⁹ reported: "While the pulmonary arteries almost regularly escape, it is not at all uncommon to find the lesion in the bronchial arteries." Barnard and Burbury²⁰ noted that the "pulmonary arteries are elastic arteries and the arteries most commonly affected in polyarteritis nodosa are muscular arteries and this may give some clue to the ætiology of the disease." In the Cabot case 25141,²¹ Mallory commented: "One pulmonary artery showed a lesion which was very suggestive of periarteritis nodosa. This is the first time I have seen a lesion in a pulmonary artery, though we have seen them in the bronchial arteries." Lichtman, Stickney, and Kernohan¹³ reported a case in which the "microscopic examination disclosed extensive arteritis of most vessels of practically every organ in the body except the retina of the eyes and lungs." In a case reported by Herbut and Price²² which presented widespread lesions of periarteritis nodosa "the vessels of the lungs and spleen were not involved." On the other hand, Ophüls, Mönckeberg, and others have reported cases in which changes in the pulmonary arteries were marked.

Many of the reported cases, with detailed microscopic descriptions of lesions, can be classified according to the criteria in this report. Without concern, at present, for the possible variations in the clinical manifestations of these two conditions, the differences in the structure and distribution of the lesions suggest a difference in cause.

SUMMARY

The various stages in the development of experimentally produced lesions of periarteritis nodosa in rats were observed, from the time of their initiation until 52 weeks later. Similar lesions were found in each of 15 cases in man. Seven other cases in man, which were associated clinically with hypersensitivity, presented vascular lesions of a different type and distribution which were designated hypersensitivity angiitis.

Periarteritis nodosa can be differentiated from hypersensitivity angiitis by (1) the morphologic characteristics of early pre-exudative le-

sions, (2) the distribution of the lesions in relation to the bifurcation of vessels, (3) the size and type of vessels primarily affected, and (4) by the presence or absence of lesions in the splenic follicular arterioles and in the arteries of the pulmonary circulation.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 142

FIG. 1. Periarteritis nodosa in the rat. Proliferative lesion, 7 days. Pancreas. Figures 1, 4, and 7 are from the same rat. Hematoxylin and eosin stain. $\times 280$.

FIG. 2. Periarteritis nodosa in the rat. Early proliferative lesion, 3-day stage. Myocardium. Hematoxylin and eosin stain. $\times 650$.

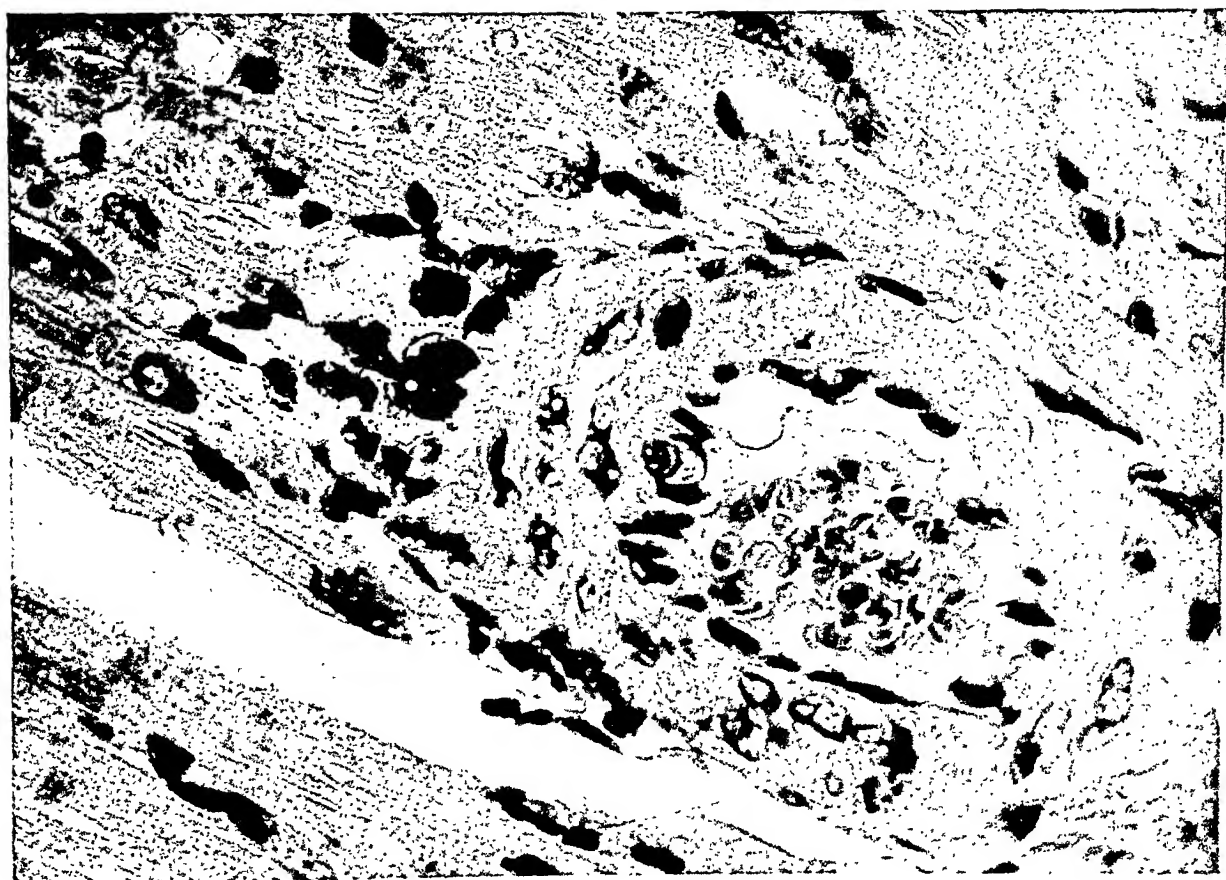
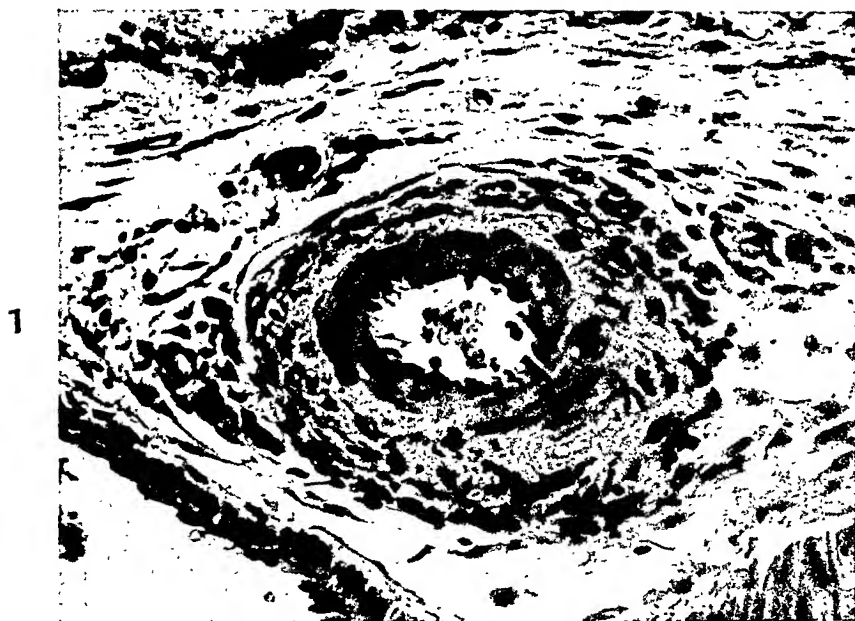
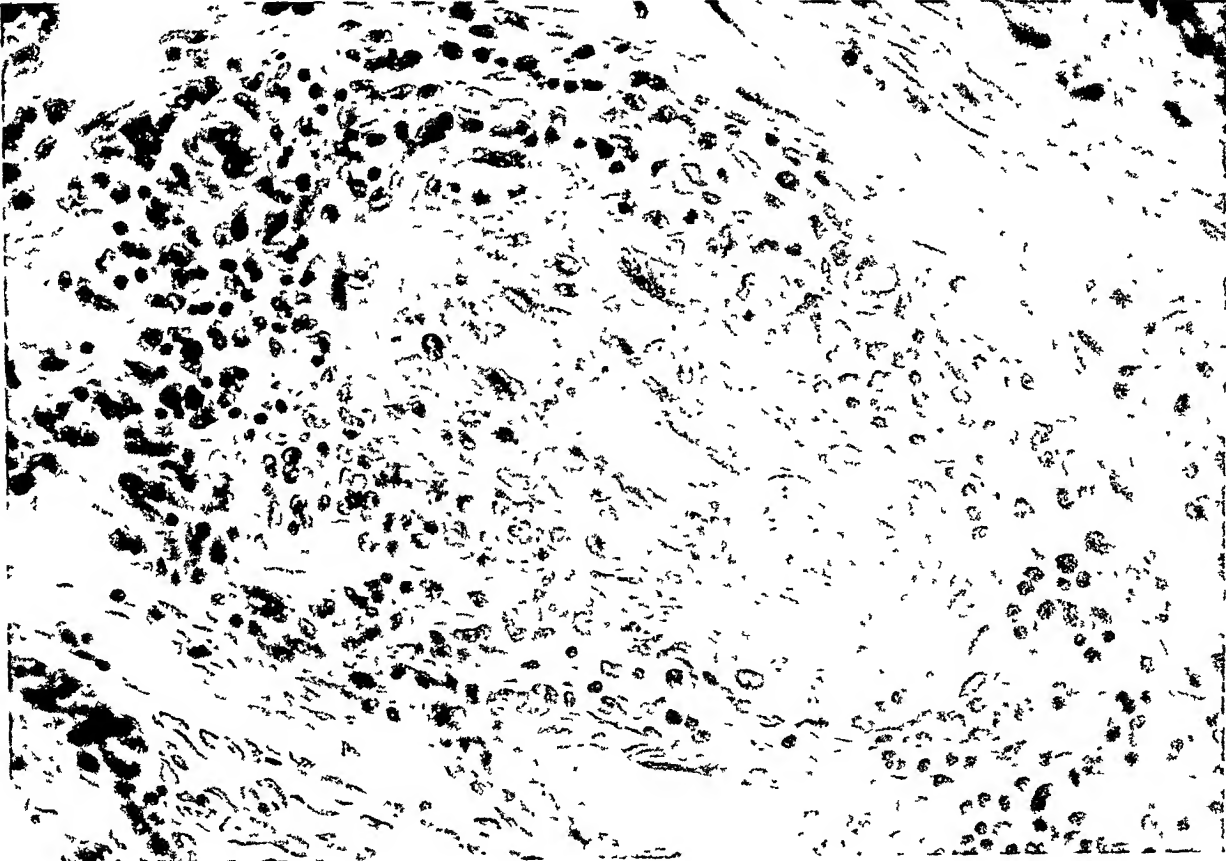


PLATE 143

- FIG. 3. Periarteritis nodosa in man. Proliferative lesion. Gallbladder. For comparison with Figure 1. Hematoxylin and eosin stain. $\times 325$.
- FIG. 4. Periarteritis nodosa in the rat. Early necrotizing lesion at the bifurcation of an artery between the pancreas and spleen, 7 days. Hematoxylin and eosin stain. $\times 160$.

3



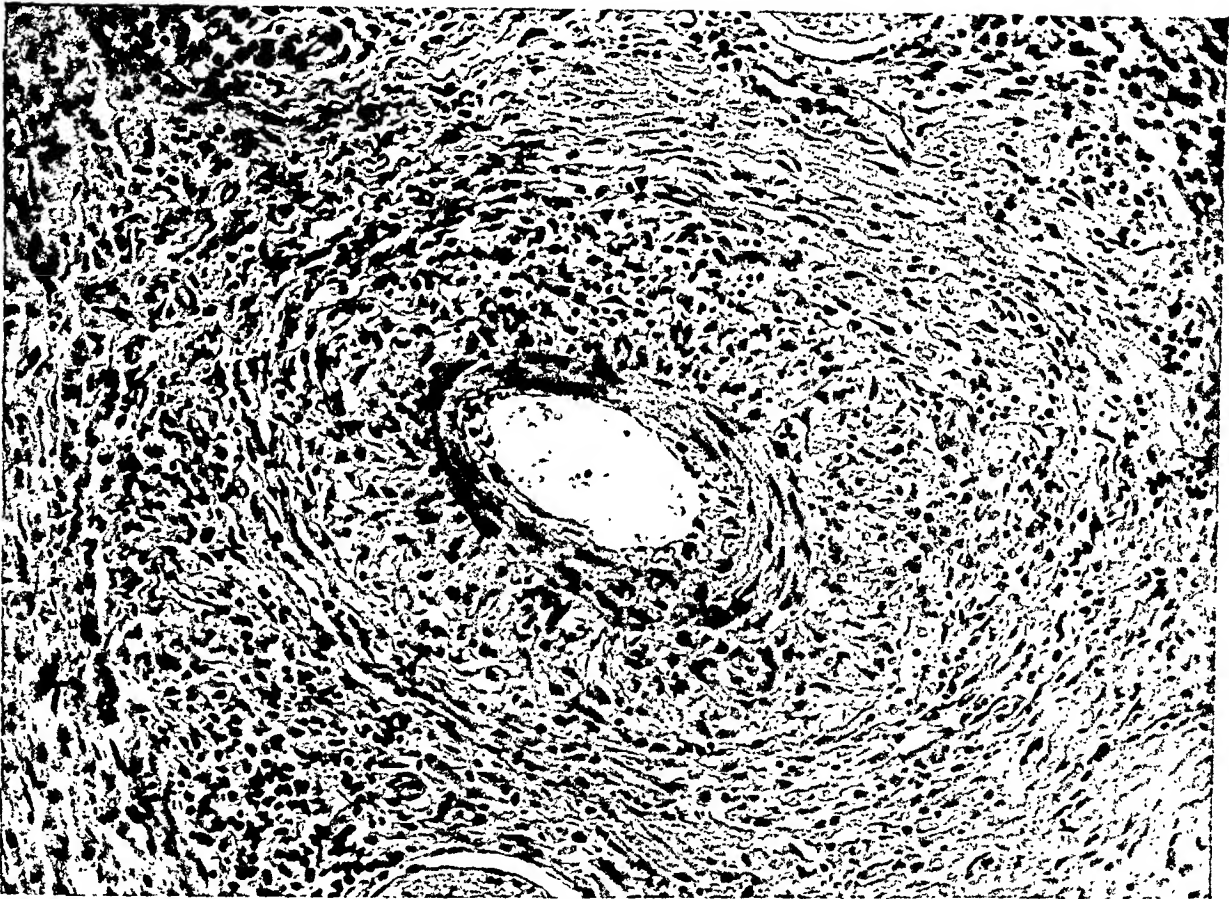
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PLATE 144

- FIG. 5. Periarteritis nodosa in man. Early necrotizing lesion in an artery near the suprarenal gland. Radiating fibroblasts and streaks of beginning necrosis are present in the media. Hematoxylin and eosin stain. $\times 160$.
- FIG. 6. Periarteritis nodosa in the rat. Necrotizing lesion in a spermatic artery, 7 weeks. Hematoxylin and eosin stain. $\times 160$.
- FIG. 7. Periarteritis nodosa in man. Necrotizing lesion in the wall of the intestine. For comparison with Figure 6. Hematoxylin and eosin stain. $\times 160$.

5



6



7



PLATE 145

FIG. 8. Periarteritis nodosa in the rat. Necrotizing exudative lesion, 7 days. Pancreas. Hematoxylin and eosin stain. $\times 160$.

FIG. 9. Periarteritis nodosa in man. Necrotizing exudative lesion at the bifurcation of a cystic artery. Hematoxylin and eosin stain. $\times 85$.

80



80

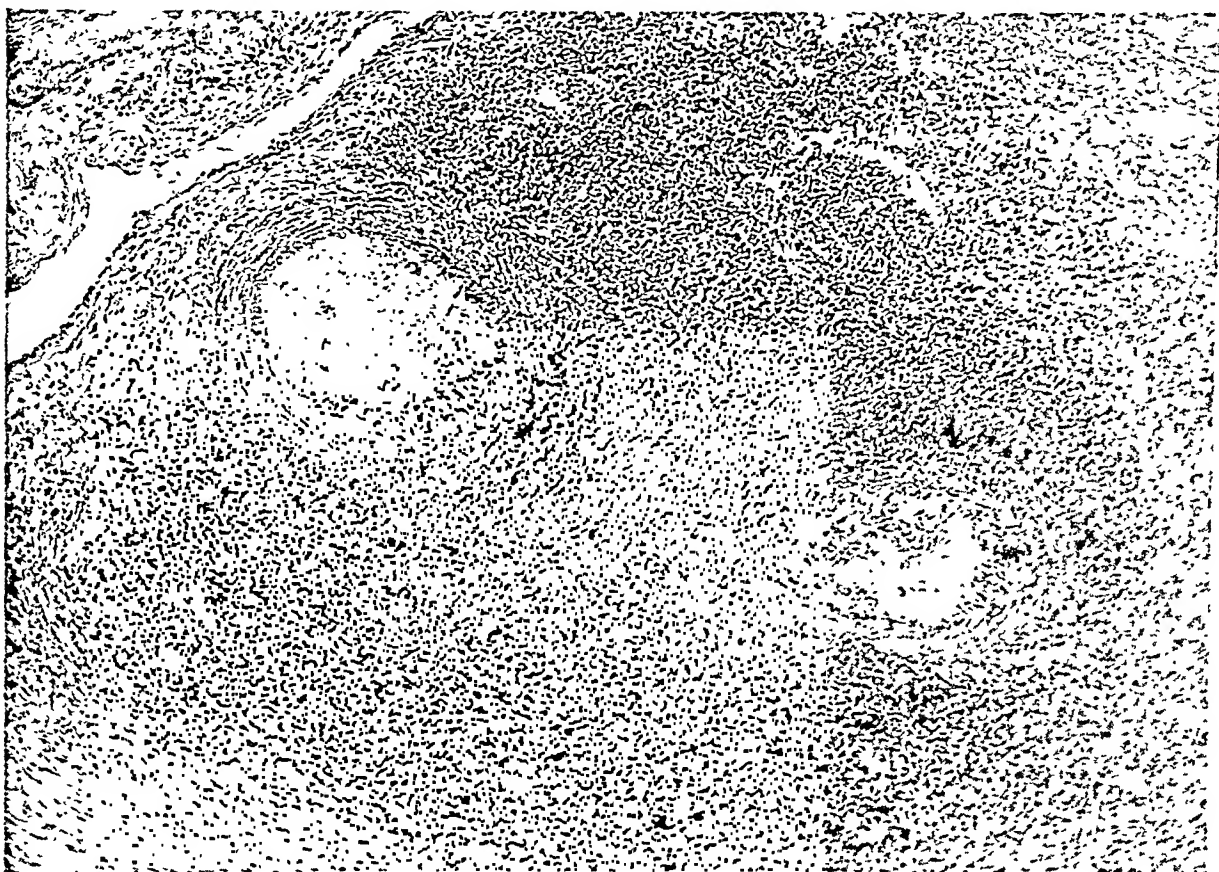
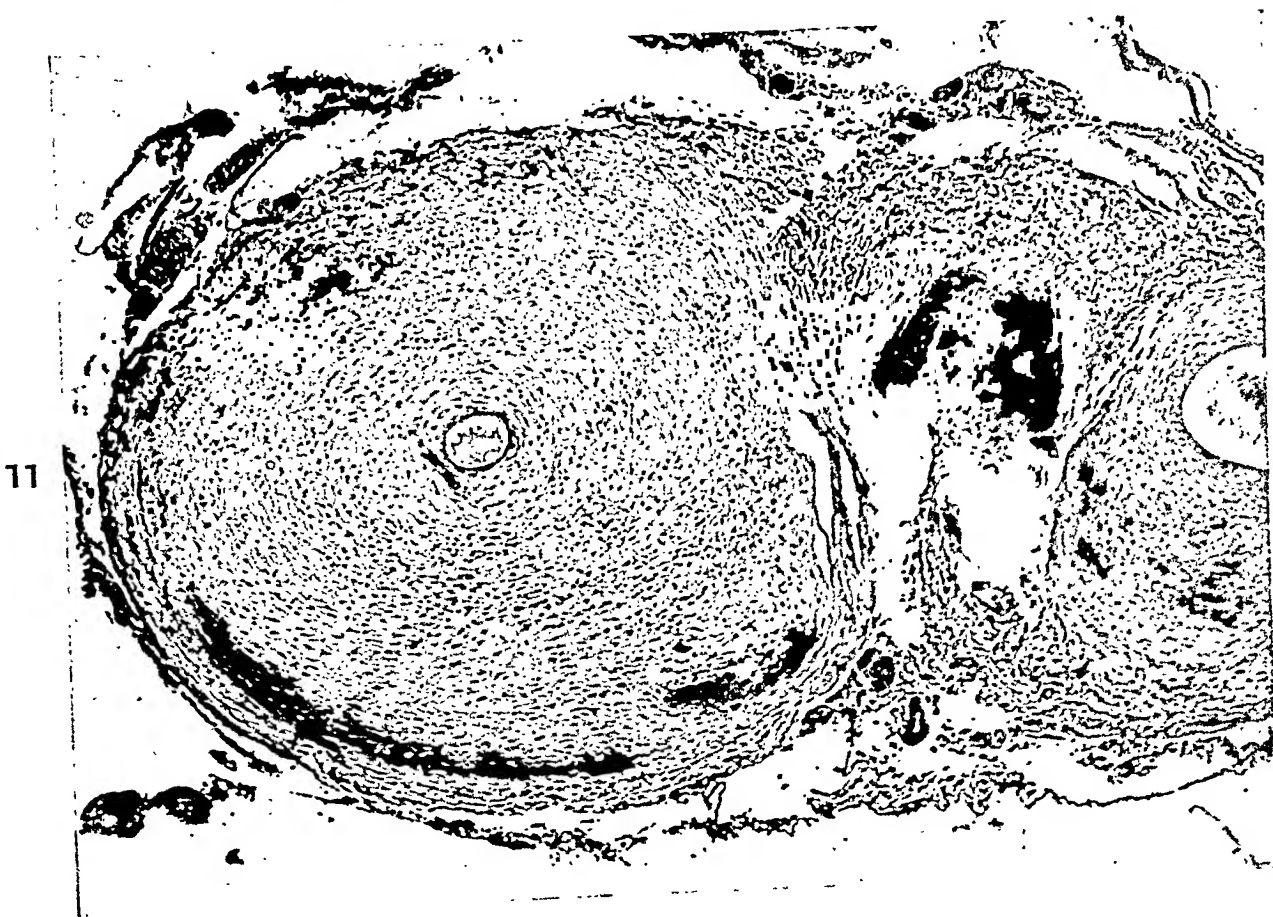
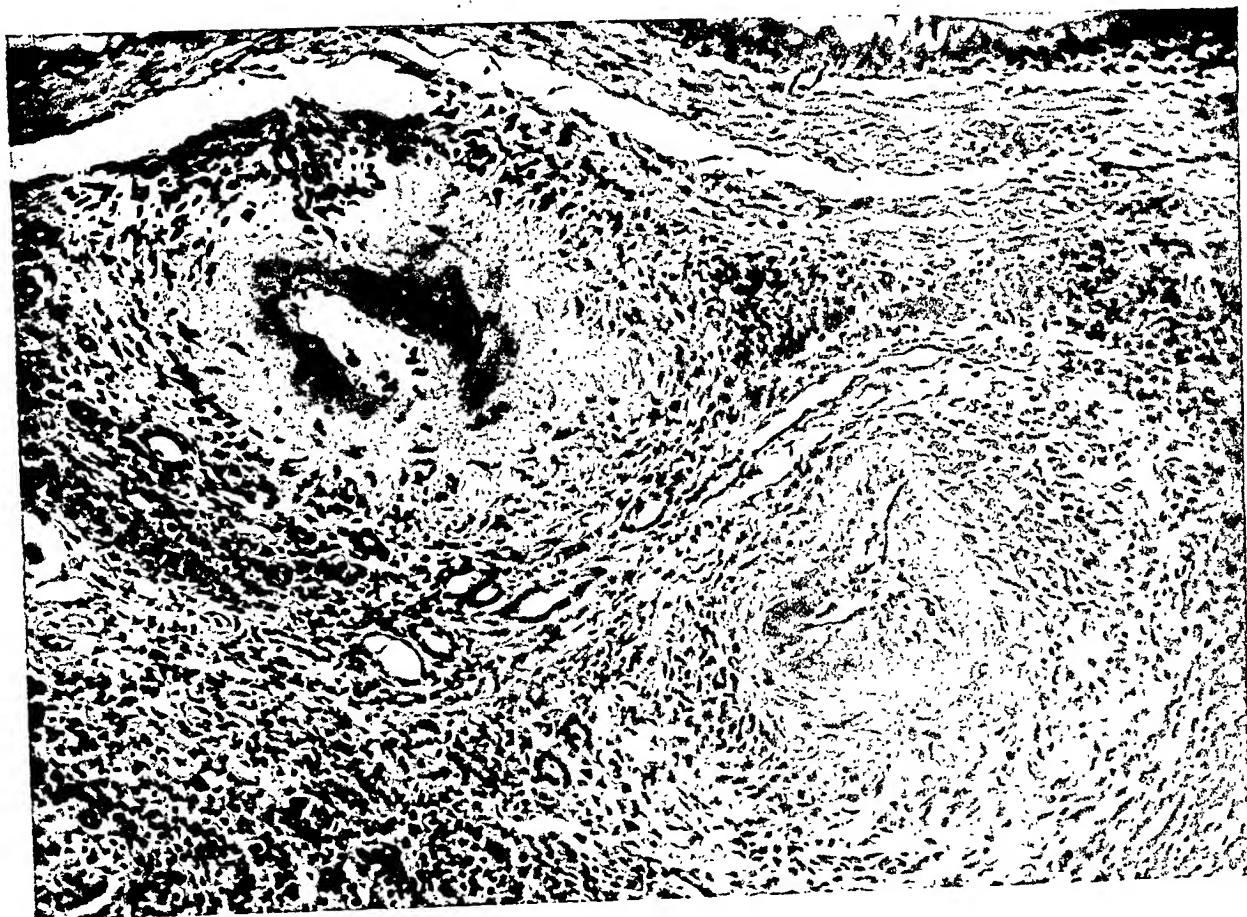


PLATE 146

FIG. 10. Periarteritis nodosa in the rat. Lesions healing by granulation tissue formation, 4 weeks. Renal pelvis. Hematoxylin and eosin stain. $\times 163$.

FIG. 11. Periarteritis nodosa in the rat. Healed, calcified lesion, 10 weeks. Splenic artery. Hematoxylin and eosin stain. $\times 58$.



Periarteritis Nodosa and Hypersensitivity

PLATE 147

FIG. 12. Periarteritis nodosa in man. Healed lesion. Coronary artery. There is localized loss of the media. Hematoxylin and eosin stain. $\times 85$.

FIG. 13. Periarteritis nodosa in man. Healed lesion. Coronary artery. Hematoxylin and eosin stain. $\times 30$.

FIG. 14. Periarteritis nodosa in man, from the same case as seen in Figure 13. Biopsy of a necrotizing exudative lesion of a gastrocnemius muscle, 47 days before death. Hematoxylin and eosin stain. $\times 160$.

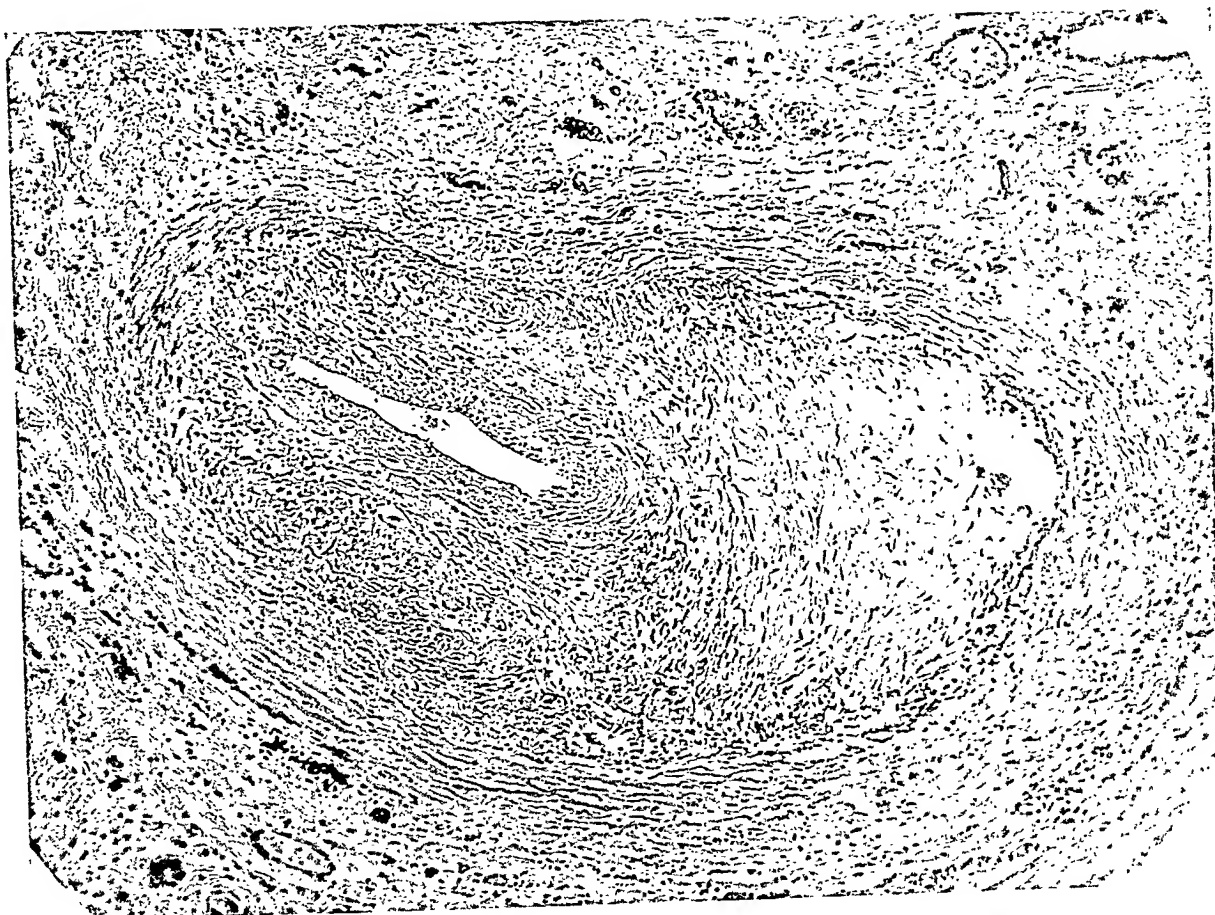


PLATE 148

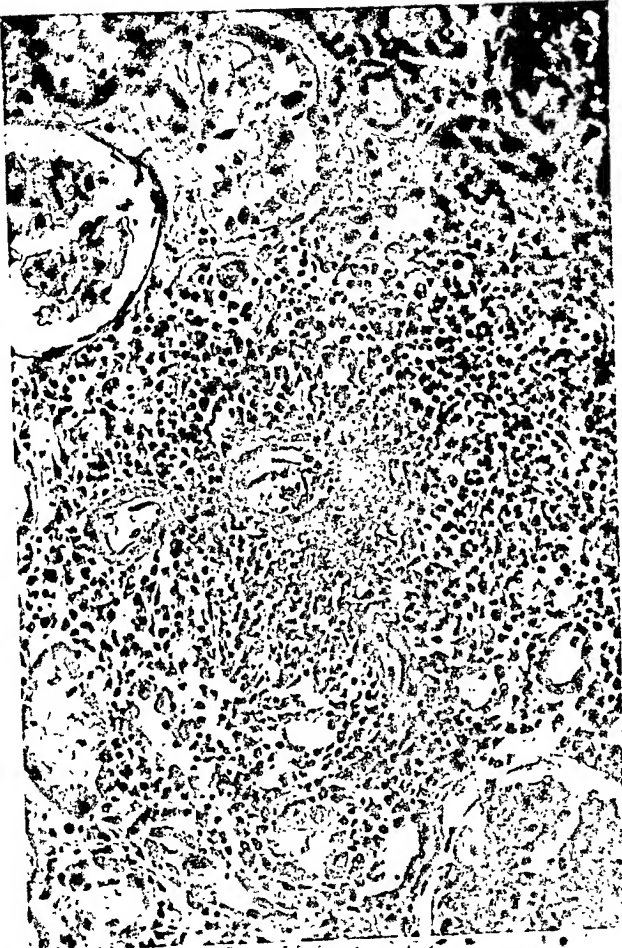
FIG. 15. Periarteritis nodosa in the rat, 15 weeks. Nodules are present in mesenteric arteries at the mesenteric attachment to the intestine.

FIG. 16. Hypersensitivity angiitis in man. Kidney. Hematoxylin and eosin stain. $\times 160$.

FIG. 17. Hypersensitivity angiitis in man. Splenic follicle. Hematoxylin and eosin stain. $\times 160$.

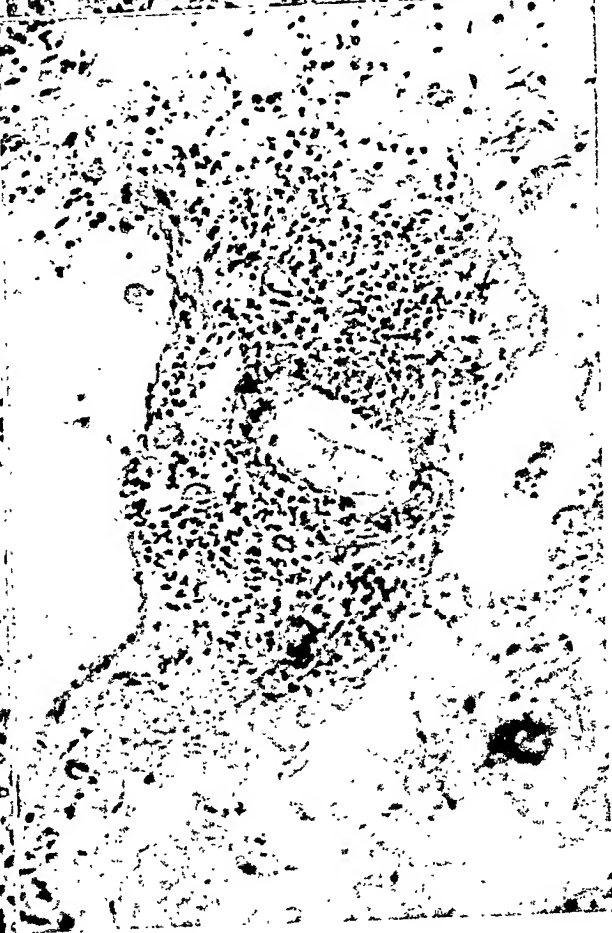
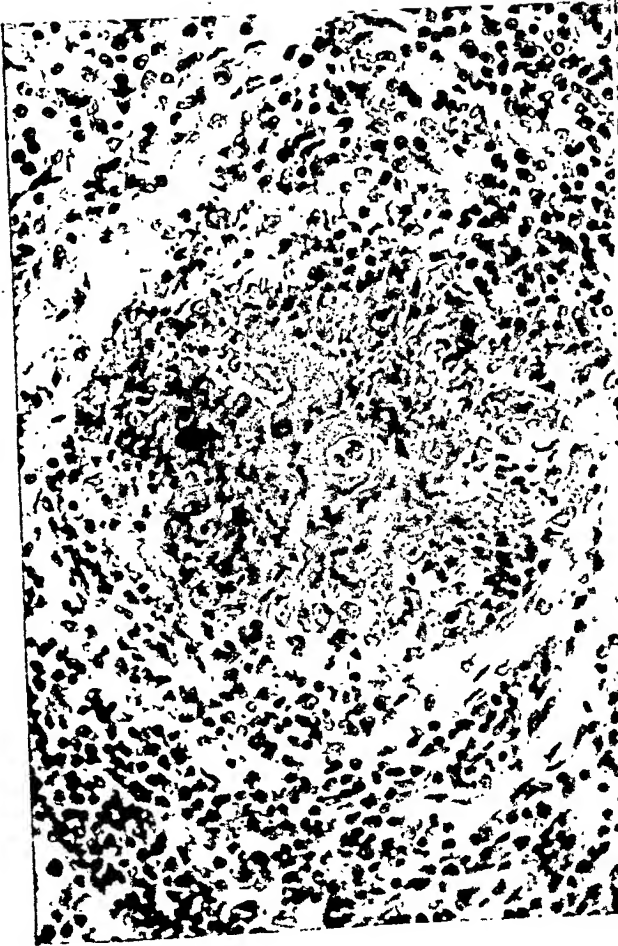
FIG. 18. Hypersensitivity angiitis in man. Lung. Hematoxylin and eosin stain. $\times 160$.

15



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17



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PLATE 149

FIG. 19. Endophlebitis in a splenic trabecula, from the same case as Figure 17. Hematoxylin and eosin stain. $\times 160$.

FIG. 20. Hypersensitivity angiitis in portal vein of the liver from the same case as Figure 16. Hematoxylin and eosin stain. $\times 160$.

FIG. 21. Necrotizing diffuse glomerulonephritis, from the same case as Figures 17 and 19. Hematoxylin and eosin stain. $\times 160$.

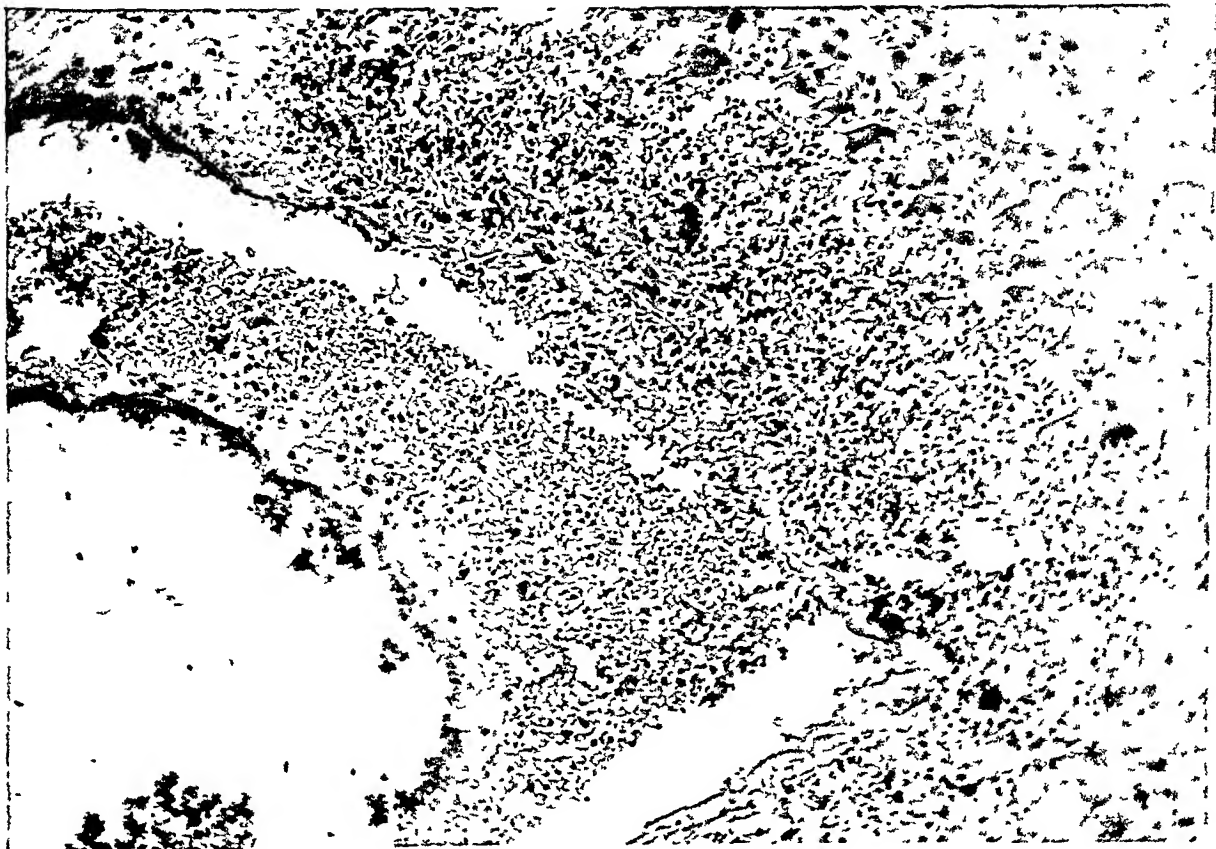
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20



CONGENITAL ALVEOLAR DYSPLASIA OF THE LUNGS *

H. EDWARD MACMAHON, M.D.

(From the Department of Pathology and Bacteriology of Tufts College Medical School, Boston, the Boston Floating Hospital and Mount Auburn Hospital, Cambridge, Mass.)

The purpose of this paper is twofold: first, to describe an interesting morphologic anomaly of the lungs which has been referred to as congenital alveolar dysplasia,¹ and second, to distinguish this anomaly from fetal atelectasis with which it has constantly been confused. Congenital alveolar dysplasia is characterized anatomically by a defective and hypoplastic development of pulmonary alveoli. In an extremely severe case there are not enough alveoli to sustain life, and death within the first 48 hours results. Clinically, the story is that of a full-term child showing respiratory distress and progressive and intractable cyanosis. The correct diagnosis in such a case is usually fetal atelectasis, since it is generally acknowledged that this condition, namely, fetal atelectasis, is often responsible for just such a clinical picture. What has not been generally recognized—and this seems important—is the fact that a primary congenital anomaly of pulmonary alveoli also can produce the same clinical syndrome.

There has been no attempt in practice to separate cases showing this anomaly or malformation from those associated with atelectasis, and for this reason it seems justifiable to consider what the terms "atelectasis" and especially "fetal atelectasis" imply. Atelectasis means an alteration in the normal physical state of lung tissue in which healthy pulmonary alveoli show varying degrees of collapse. This is accompanied by a relative increase in the capillary bed and, because of defective ventilation in the involved portion of lung, the area becomes a deep cyanotic red. Atelectasis is often complicated by such local disturbances in circulation as congestion, edema, and hemorrhage. There may be superimposed infection and, in the case of the newborn, atelectasis may be partly obscured by the aspiration of amniotic fluid. When free from these complications, atelectasis is a very simple and reversible pathologic change.

Atelectasis may be induced in a number of ways. When healthy lung parenchyma is compressed, it is known as *compression atelectasis*. If a bronchiole is completely obstructed, the air distally becomes resorbed

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and the alveoli collapse. This is referred to as *obstruction atelectasis*. A third type that is also associated with the resorption of residual air is seen in children who are very ill and show a very limited respiratory excursion. Under these circumstances, the portions of collapsed lung tissue are usually confined to the paravertebral areas. Atelectasis of this type is likely to be bilateral and nearly symmetric. It has been suggested that this form of atelectasis may at times represent a terminal process, since it is found more commonly at autopsy than is suspected during life. Because this is usually associated with weak and shallow breathing, it may be designated as *hypopneic atelectasis* to distinguish it from other forms. There is a fourth category, and this is seen when normally developed lung parenchyma of a premature or full-term child fails to expand. This is commonly referred to as *fetal atelectasis*. It is a frequent finding in the newborn at the autopsy table, and is a well recognized basis for respiratory failure, asphyxia, and death. In such cases, it is the duty of the pathologist to search for all factors responsible for this atelectasis since it must be remembered that fetal atelectasis is not a primary structural deformity, but only an alteration in the normal physical state in which, for one or a number of reasons, normally developed and expandable alveoli have failed to dilate. Farber and Wilson,² in an excellent review and discussion of atelectasis of the newborn, have referred to still another form. This, they have pointed out, is found in all premature infants. The extent of this form of atelectasis is simply proportional to the degree of prematurity of birth. It is characterized by the presence of areas of unexpanded, developing pulmonary tissue in the form of solid end-knobs of epithelial cells, known as pneumoneres, which are incapable of expanding. It is debatable whether one should refer to such areas as atelectatic since this term usually implies that the involved alveoli are already developed and capable of expansion.

In contrast to these varied forms of atelectasis, *congenital alveolar dysplasia* may be considered a primary malformation of pulmonary alveoli, the pattern of which suggests a severe retardation in normal alveolar development. Its etiology is obscure. It varies in extent and in degree. It may involve both lungs uniformly or only a portion of a single lobe. When the lesion is minimal, even though diffuse, it is compatible with life, but when the changes are severe, it leads to early death. It apparently has its origin in early embryonal development, probably dating back as far as the tenth or twelfth week of intrauterine life. It has been found in identical twins and it has been encountered with and without developmental anomalies in other organs.

Gross Findings

Lungs of mature newborn children showing diffuse pulmonary alveolar dysplasia appear well formed. They are large as though distended, and each lung fills its corresponding pleural cavity. The lungs are firm, rubbery, and dark red. There is little or no crepitation and they seem almost airless. On placing the whole lung in water, it sinks slowly or remains suspended below the surface. Minute snippings vary in this respect, some sink and others float. Each lung weighs about 10 gm. more than is normal for the newborn child. The lungs are fleshy and may be easily cut in narrow strips. The freshly cut surfaces are dark red and little fluid may be expressed on pressure. In this fresh state, and without freezing or any form of fixation, the lungs may be immediately examined microscopically, allowing a more accurate diagnosis to be made. Utilizing a rapid section technic,³ thin sections cut with a razor blade may be prepared by drawing a dry slide, previously stained with 1 per cent toluidine blue, across the freshly cut surface. Such preparations will reveal an excess of interstitial tissue that is rich in capillaries. This surrounds a few isolated and sometimes overdistended pulmonary alveoli.

It must be admitted that such lungs may resemble those showing diffuse atelectasis complicated by congestion or edema, or by the aspiration of amniotic fluid. An accurate gross diagnosis of any pulmonary disease in the newborn child may be difficult, and because some are so similar, the less common and less conspicuous are often overlooked.

Histologic Findings

The most striking change involves the alveolar spaces and their walls. There are too few alveoli and there is far too much interstitial tissue (Fig. 1). Some of these spaces are very small, while others are so abnormally distended as to suggest congenital alveolar ectasia (Figs. 2 and 3). Most of the alveoli are empty, but some contain an eosinophilic material (Fig. 4) that varies in texture from hyaline to granular. This substance clings to the surface of the alveoli like an inner lining, and although few disintegrating cells are enmeshed in this substance, it contains no cornified cells to suggest that it results from the aspiration of vernix. Nests of flattened epithelial cells and numerous dilated capillaries provide an inner surface for the alveoli. There is no demonstrable basement membrane limiting their walls and much of the inner surface appears to be bare of epithelium. Their walls are many times thicker than is normal and this accounts for an almost complete reversal in the ratio of air space to stroma (Fig. 5). The walls are

composed of mesenchymal tissue of an embryonal type and an exceedingly rich network of dilated capillaries. This mesenchyme, in turn, is made up of an abundance of relatively undifferentiated fibroblasts held loosely together by a scarcely detectable intercellular ground substance. Sections prepared with Mallory's acid fuchsin-aniline blue stain show well formed collagen fibrils in the pleura, in the interlobular septa, and about the larger vessels and bronchi, but fail to reveal a network of mature collagen in the thick walls of the alveoli. Usually collagen is readily demonstrable in the walls of normally developed alveoli. The material noted earlier in some of the alveoli assumes varying shades of red with this connective tissue stain but it lacks the brilliance and fibrillation of fibrin. No elastica is demonstrable in the walls of the alveoli in this condition, but this is not surprising since elastic fibrils are very seldom found at this early age in such areas in normally developed lung tissue.

A second, but less constant and less conspicuous finding in the lungs showing congenital alveolar dysplasia is an exaggerated demarcation of the lobules by abnormally wide interlobular septa. Broad edematous tracts of loose connective tissue composed of few fibroblasts, little collagen, dilated capillaries, and lymphatics separate one lobule from another. Occasionally, small islands of hematopoietic tissue are found in the interalveolar mesenchymal tissue.

This rather simple picture of a lung showing too much stroma and too little air space may be complicated by congestion, edema, and hemorrhage. Such lungs may also show aspiration of amniotic fluid or infection with subsequent inflammation. In addition there may be small foci of atelectasis. Any of these complications may obscure an underlying congenital anomaly involving the alveoli and their walls.

Clinical Findings

The clinical findings in congenital alveolar dysplasia can best be studied by considering the records of 3 typical cases. Each of these had been diagnosed clinically as fetal atelectasis, and in subsequent clinicopathologic conferences this same diagnosis was consistently upheld by all physicians who entered into the discussion. My attention was first drawn to this condition in the course of an autopsy on a full-term male infant who died on the day following delivery.

A 1-day old infant was referred to the Boston Floating Hospital* because of cyanosis and respiratory difficulty. The pregnancy had not been unusual and birth was said to have been normal. The baby breathed and cried spontaneously and for several hours nothing unusual was observed. After about 12 hours, it became

*For the clinical record of this case I am indebted to Dr. James Marvin Baty, Physician-in-Chief, Boston Floating Hospital, Boston, Massachusetts.

obvious that the child was breathing with difficulty and was cyanotic. On the second day, the child was critically ill. Breathing was rapid and distressed and cyanosis was constantly present. On admission, the child was immediately placed in a Hess bed and oxygen was administered constantly. Respirations became more rapid, reaching 100 per minute. The child expired 36 hours after delivery. Physical examination 6 hours before death revealed a very sick child weighing 6 lbs. The cry was weak and there was very severe cyanosis of all ectodermal protective layers. Respirations were extremely rapid and there was marked substernal and intercostal retraction. There was dullness over both sides of the chest and breath sounds were diminished. The remainder of the physical examination was not unusual. The clinical diagnosis was fetal atelectasis.

Autopsy (A-45-3-B.F.H.) revealed a mature child with well developed epiphyseal bone nuclei at the end of each femur. Both lungs showed the gross and histologic findings of congenital alveolar dysplasia. In addition, the autopsy showed a number of other anomalies including an almost complete defect in the interauricular septum of the heart, multiple valvular hematomata, and hypoplasia of the brain with scattered foci of spongioblasts beneath the ependymal covering of the ventricles.

A second case * was encountered about a year later.

During the early course of pregnancy, the mother (para II) had not been well. She had had pyuria for which she had been treated several times with different sulfa drugs. She was markedly anemic and had received liver injections and iron by mouth without much benefit. In addition she had repeated colds and, for a long period, would take no fruit juice. About the third month there had been some staining, necessitating continuous bed rest. She was admitted at term for an elective cesarean section. The operation was uneventful and the mother made an early recovery. The baby had a strong cry and breathing began immediately. When taken to the nursery, the child appeared to be breathing normally, but about an hour later there were obvious signs of respiratory distress. Coramine was then given and oxygen administered continuously. Breathing became more rapid and cyanosis increased. The child expired 36 hours after delivery. The clinical diagnosis, supported by roentgenologic findings, was fetal atelectasis.

Autopsy (A-46-82-M.A.H.) revealed diffuse congenital alveolar dysplasia of both lungs. The child, a male, was fully mature and weighed 6 lbs. One other morphologic anomaly was a small accessory spleen.

A third case,† again about a year after the last, was also diagnosed during life as fetal atelectasis.

The mother was admitted a few days before term for an elective cesarean section. The postoperative course was uneventful. A 6½ lb. female child was delivered readily. The child cried promptly and breathing seemed normal. Fifteen minutes later it was noted that the child was doing poorly. There was diaphragmatic breathing and moderate cyanosis. Physical examination revealed almost complete absence of breath sounds bilaterally. The child was placed immediately in an incubator and given continuous oxygen. Two hours after delivery, when first examined by a pediatrician, it was found to be ashen and blue. Its respira-

* For the clinical history of this case, I am indebted to Dr. A. J. D'Elia of the Cape Cod Hospital, Hyannis, Massachusetts.

† For permission to use the clinical notes of this case, I am indebted to Dr. H. V. Hyde of the Mount Auburn Hospital, Cambridge, Massachusetts.

tions were grunting. It had no cry and it was distinctly hypotonic. Both lungs were flat to percussion. After the administration of oxygen, the child's color and cry improved and for a time it became more active. About 36 hours later, respirations were very rapid and shallow, and cyanosis progressively increased. The child ceased breathing 48 hours after birth. The clinical diagnosis was fetal atelectasis.

At autopsy, a gross diagnosis of probable congenital alveolar dysplasia was made on the lungs, and this was confirmed immediately by rapid section examination.³ The child was well nourished and the skeletal and muscular systems were well developed. In addition to the anomaly in the lungs, there was a small accessory spleen, and there was a valve-like constriction in the upper end of each ureter near its junction with the renal pelvis.

A comparison of the clinical records of these three full-term infants, who died as the result of a developmental malformation of the lungs, clearly reveals a striking similarity in all. Each child cried and breathed promptly at birth and, for a period varying from minutes to hours, there was no apparent impairment in respiration. Later, breathing became progressively distressing and cyanosis became increasingly obvious. The chest findings were dominated by flatness to percussion and by diminished to absent breath sounds. All three children expired within a period of 36 to 48 hours after birth, and in each case, it must be emphasized, the clinical diagnosis was fetal atelectasis of unexplained cause.

DISCUSSION

If one accepts the definition of atelectasis as being simply the collapse of normally developed, but expandable lung tissue, there seems to be no justification for considering these three cases as belonging to that category of pulmonary disease. The question then naturally arises: What is the nature of the changes in the lungs of these children that, in this severe and diffuse form, has led to their death?

At first glance under the microscope the lesion suggests a non-specific, proliferative, interstitial pneumonitis, or an interstitial form of congenital syphilis of the lungs, but in neither parents nor infants was there any evidence of syphilis nor, after a more careful examination, was there any sign of an inflammatory disease. The histologic structure of the lung bears a resemblance to the pattern in a 3 to 4-months-old fetus (Fig. 6). At that stage, it will be recalled, the immature lung is unusually rich in mesenchyme. This similarity between an immature lung and congenital alveolar dysplasia suggests that the latter is merely a manifestation of extreme retardation in alveolar development, but the histologic picture of the lung in congenital alveolar dysplasia is not the same as that of an early fetal lung. There are

several differences. First, the capillary bed in alveolar dysplasia is infinitely greater than that of early embryonal life. Secondly, the alveolar epithelium in the relatively few alveoli resembles that of the mature lung far more closely than it resembles the cuboidal gland-like epithelium of the embryo or fetus. Thirdly, the bronchial epithelium in congenital alveolar dysplasia is well developed and resembles that of a full-term child. Lastly, there is an unevenness in the distribution of both alveoli and interstitial tissue that is not found in early fetal life. In one particular respect, however, the lungs in congenital alveolar dysplasia resemble very closely the lungs of a much earlier period. This point is nicely revealed with the aid of the Mallory connective tissue stain. The alveolar walls are not only abnormally wide as in the immature lung, but they are also composed of a primitive mesenchyme devoid of mature collagen fibrils. There is no question of total hypoplasia or diminished growth of the lungs since, in respect to both size and weight, these organs in congenital alveolar dysplasia are quite as large or larger than those showing normal growth. To designate this lesion, the term *congenital alveolar dysplasia* has been used, since it appeared to be a manifestation of a disturbance in the normal development of pulmonary alveoli.

The functional significance of congenital alveolar dysplasia is obvious, particularly when it is diffuse and severe. Not only are there insufficient alveoli for adequate respiration, but also, and this is equally important, the malformed lung is a relatively nonresilient organ and consequently is incapable of normal expansion and contraction. As a result, this lesion may lead to death, or, in its less severe form, while compatible with life, may explain some of the difficulties in breathing and tendencies toward repeated pulmonary infection that are seen during the first weeks or months of life. While the importance of this anomaly to the infant is quite clear, it seems worth pointing out that its recognition may be of considerable moment to the obstetrician, anesthetist, and pediatrician as well, for it is in cases of this type that the entire responsibility for the death of a child is likely to be placed on the shoulders of those who have handled the case. It might afford some measure of consolation to the parents to know that death had been the result of a developmental anomaly and not because of some error or omission in managing the case.

With reference to the three infants whose histories have been included in this paper, all were born at approximately full term. It will be apparent, however, that this same malformation may be equally well recognized in the premature child since, from the seventh month at

least, if the lung has developed normally, the alveoli have already acquired the pattern and character that is found in a healthy full-term child.

It must be emphasized that no attempt has been made in this paper to belittle or disprove the importance of fetal atelectasis, but rather to point out that a developmental anomaly of the lungs, although much less common, may produce a strikingly similar clinical picture. If one is willing to accept the fact that congenital alveolar dysplasia is a reality, there is good reason to believe that clinical and roentgenologic means will be found to diagnose it.

SUMMARY

Congenital alveolar dysplasia is proposed as an appropriate designation of a morphologic anomaly of the lungs of newborn children. It is suggested that this anomaly represents a retardation and disturbance in the normal development of pulmonary alveoli. Three cases have been included. The etiology at present is obscure.

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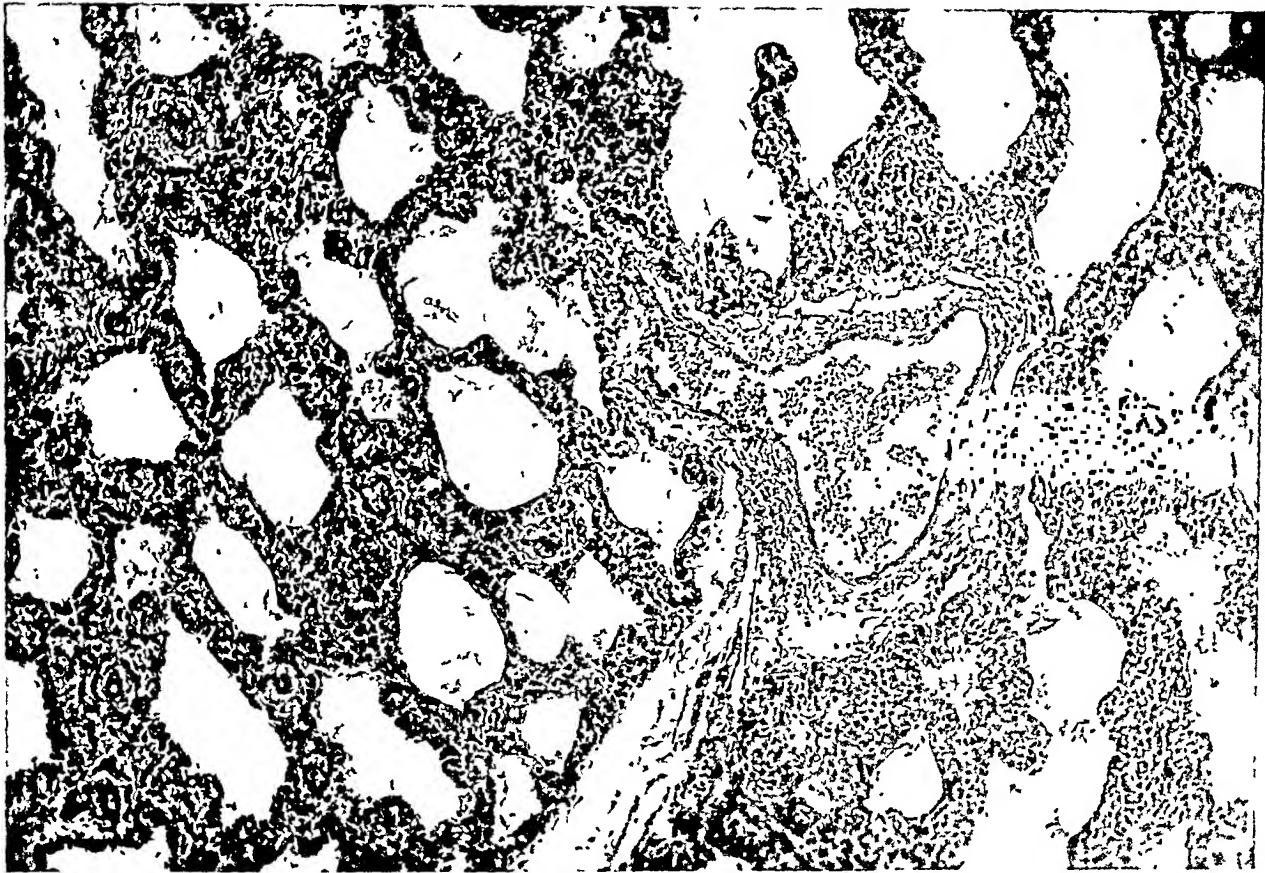
DESCRIPTION OF PLATES

PLATE 150

FIG. 1. Lung: congenital alveolar dysplasia. There are too few alveoli and there is far too much interalveolar mesenchymal stroma. Eosin and methylene blue stain. $\times 90$.

FIG. 2. Lung: congenital alveolar dysplasia. This field was selected to show the pathologic dilatation of some of the alveoli. Even in areas of maximal dilatation the alveolar walls are several times as thick as is normal. Eosin and methylene blue stain. $\times 90$.

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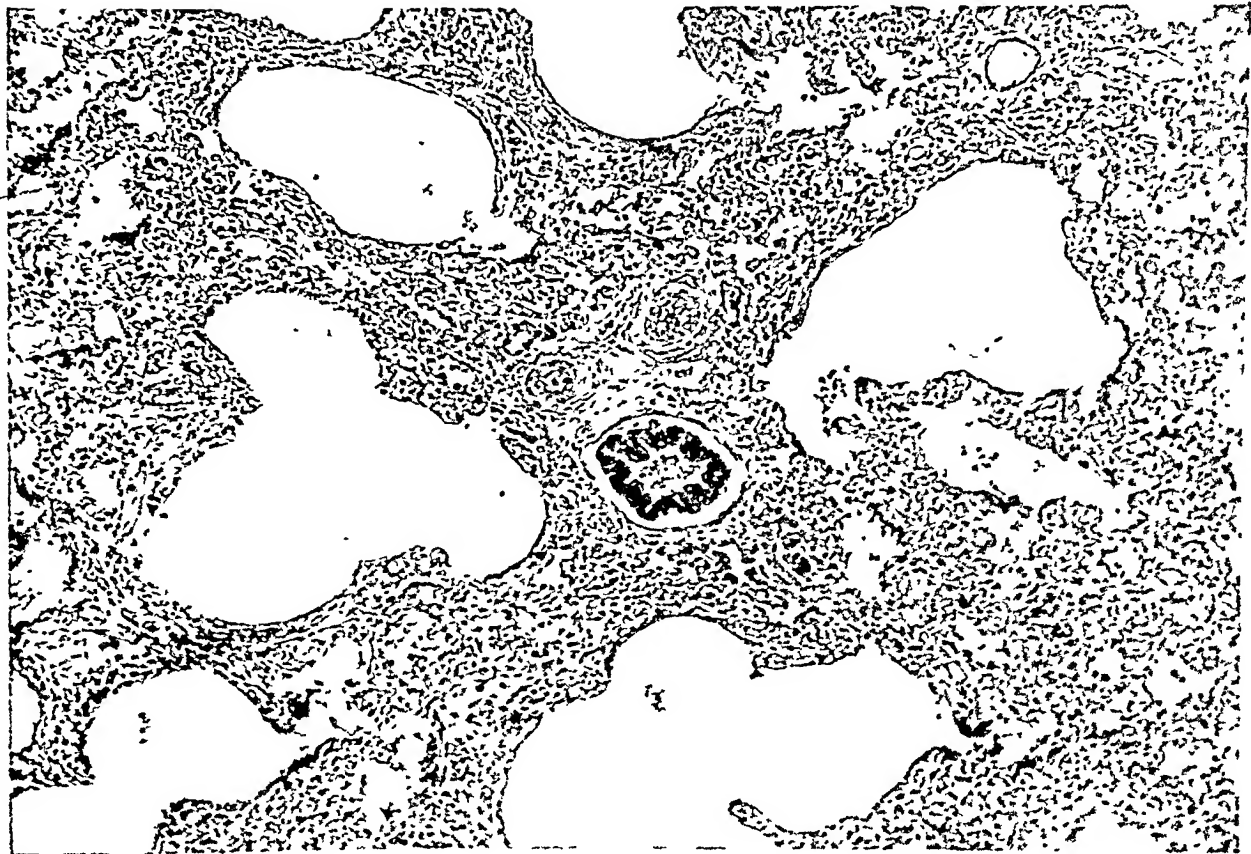
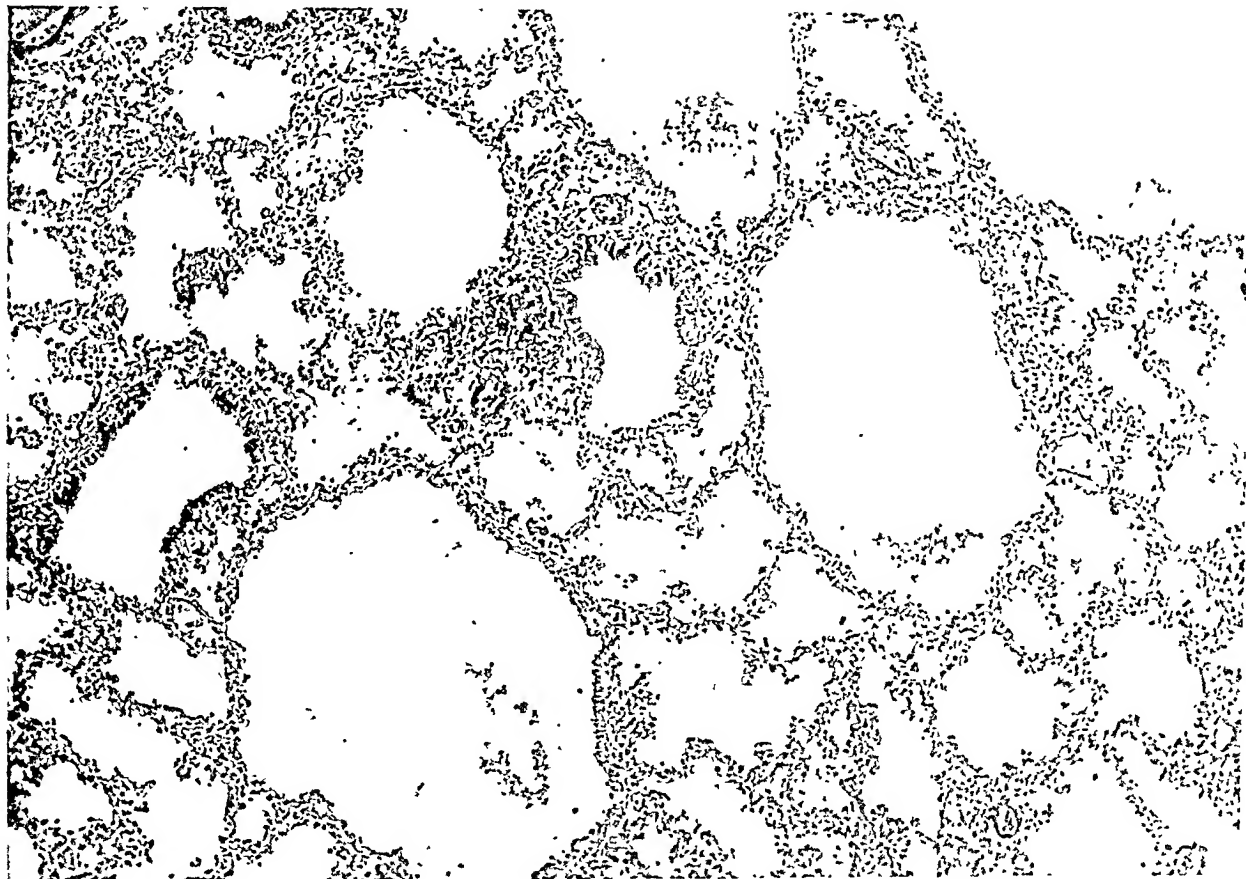


PLATE 151

FIG. 3. Lung: congenital alveolar ectasia. This is an unusual developmental anomaly that was confined to the lower left lobe of an otherwise normally developed lung of a full-term infant. This field was selected to demonstrate this malformation and to compare this primary congenital dilatation with the acquired dilatation of alveoli that may be found in congenital alveolar dysplasia (Fig. 4). Eosin and methylene blue stain. $\times 90$.

FIG. 4. Lung: congenital alveolar dysplasia. This field was selected to show considerable dilatation of some of the alveoli, the persistent excess of inter-alveolar stroma, and the accumulation in some of the alveoli of a precipitate that tends to cling to the inner surfaces. Eosin and methylene blue stain. $\times 90$.

3



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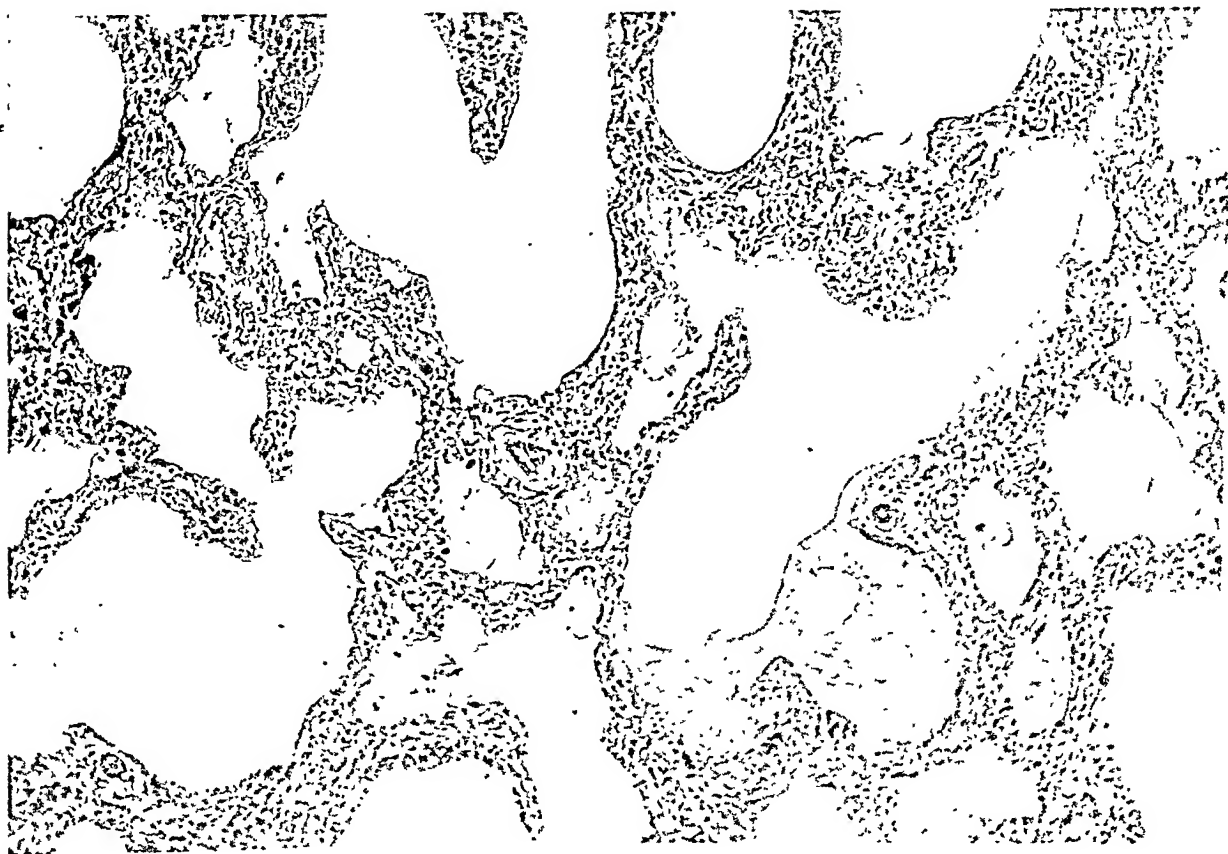
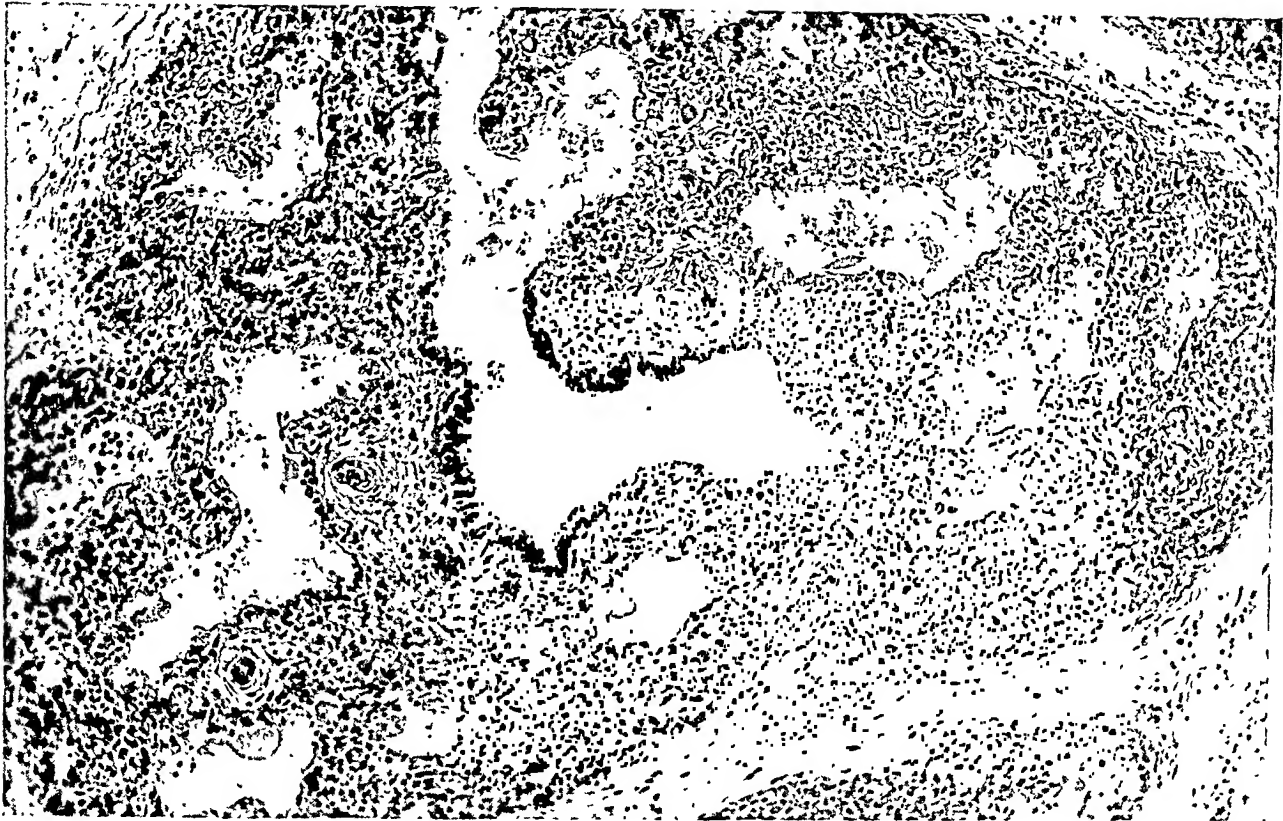


PLATE 152

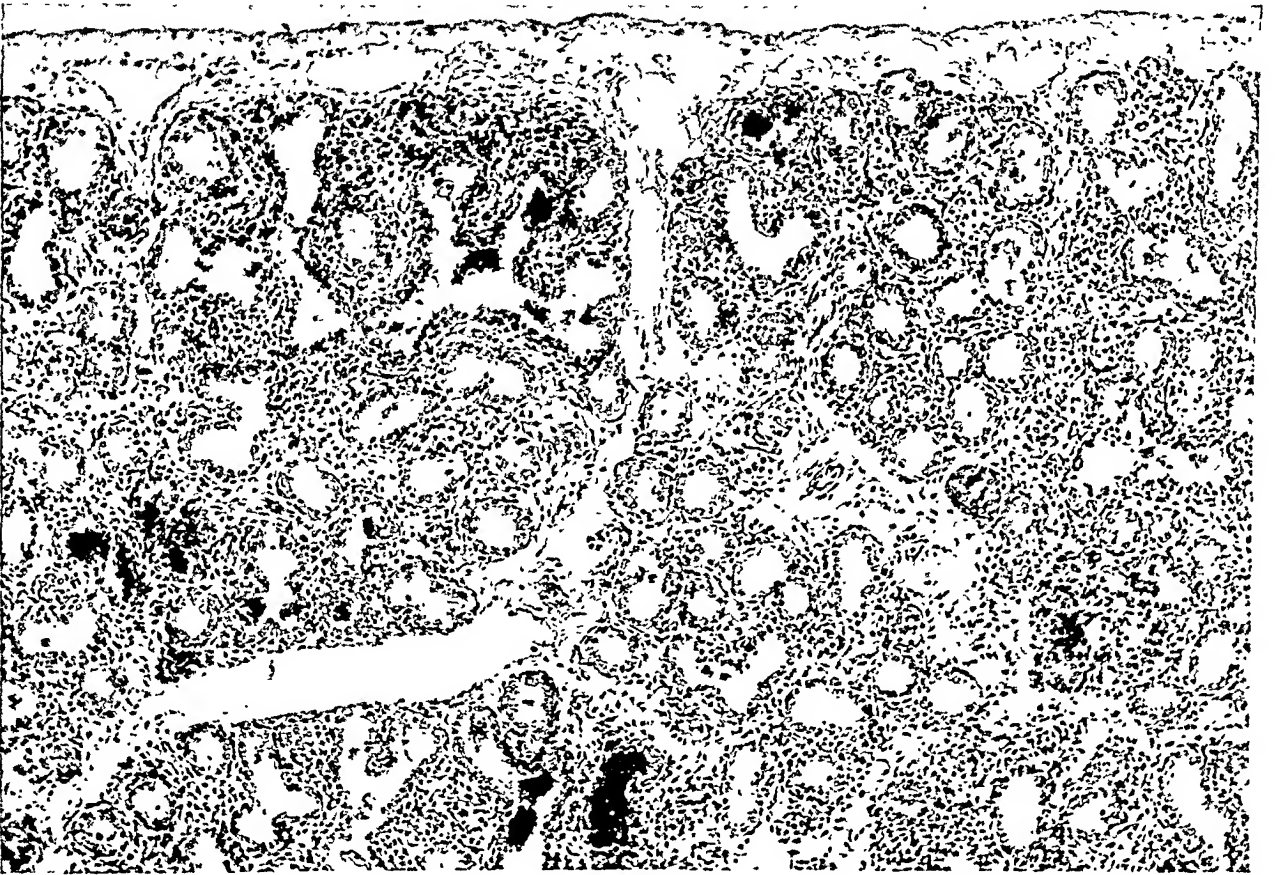
FIG. 5. Lung: congenital alveolar dysplasia. This field was selected to show the relative disproportion between the alveoli and the interalveolar mesenchyme, the vascularity of the stroma, and the accentuation of the interlobular septa. Eosin and methylene blue stain. $\times 130$.

FIG. 6. Lung: fetus, fourth month. This section was selected for comparison, to show the lung of a 4-months-old fetus. The ratio of alveoli to stroma resembles that of congenital alveolar dysplasia in a full-term infant. In this section the alveoli appear as uniformly distributed, gland-like spaces and the stroma is relatively avascular compared to that of congenital alveolar dysplasia. Eosin and methylene blue stain. $\times 130$.

5



6



ADENOMATOID TUMORS OF THE FALLOPIAN TUBE*

ALEX B. RAGINS, M.D., and ROBERT D. CRANE, M.D.

(From the Hektoen Institute for Medical Research and the Department of Pathology of the Cook County Hospital, Chicago, Ill.)

Over a period of 19 years, 7,485 fallopian tubes have been examined at the Cook County Hospital; of these, 3, or 0.04 per cent, presented tumor growths of a rather unusual structure. Because of their peculiar histologic appearance we could not apply any established terminology to these three neoplastic lesions of the fallopian tube. The first suggestion as to what we might be dealing with was presented in two relatively recent publications (Evans,^{1,2} Golden and Ash³). Although these authors approached the cytologic interpretation of the tumor from different viewpoints, we feel that they have established a sound foundation which will enable a more accurate interpretation of this tumor. It is the purpose of this paper to present three such tumors arising in the wall of the fallopian tube, thus making a total of six reported cases.

REPORT OF CASES

CASE I

O. E., a married colored woman, 45 years old, entered the Cook County Hospital (no. S-5429-37) on November 8, 1937. In August, 1937, she had noticed a mass in the lower abdomen, with almost constant vaginal bleeding. In September the patient had a severe hemorrhage which lasted about 1 week, leaving her in a very weakened condition. Between the periods of bleeding the patient experienced severe leukorrhea.

Menses had begun at the age of 14 years. Her last regular menstrual period was in 1934. At the time of this examination she complained of metrorrhagia and menorrhagia.

Physical examination revealed a well developed, well nourished woman. The temperature was 99.2° F., the pulse 90, and the respirations 20. The blood pressure was 188/98 mm. Hg. The heart was slightly enlarged to the left. A large movable mass, the size of a grapefruit, was palpated in the lower abdomen. Some tenderness was present over the symphysis pubis but there was no rigidity. On pelvic examination, Skene's and Bartholin's glands were normal and the introitus admitted 2 fingers with ease. The external cervical os was patent and soft, and the cervix was located beneath the symphysis pubis; the adnexa could not be palpated.

Wassermann and Kahn reactions of the blood were negative. On admission, the hemoglobin was 70 per cent; red blood cells, 3,900,000; white blood cells, 8,000. The urine was negative.

The following diagnoses were made: fibromyomata uteri, cancer of the cervix and corpus uteri still to be excluded, and hypertensive heart disease.

On November 19, 1937, a supracervical hysterectomy, bilateral salpingectomy, right oophorectomy, and appendectomy were performed. The patient made an uneventful recovery.

* Received for publication, July 12, 1947.

Pathologic Report

The specimen consisted of a uterus amputated at the cervix, two tubes, one ovary, and an appendix. The uterus measured 19 by 12.5 by 8.5 cm. and was deformed by intramural and pedunculated subserous nodules which measured up to 13.5 cm. in greatest diameter. On sectioning the nodes were light yellow-gray to brown-gray and distinctly trabeculated. The brown-gray areas were softened and in places friable. The endometrium measured 1 mm. in thickness and was purple-gray.

The fimbriated end of one fallopian tube was patent; the wall was thin; the mucosa a light purplish gray. The adjacent ovary measured 3.5 by 2.5 by 1.7 cm. and contained several cysts up to 20 mm. in diameter filled with a clear fluid. There were numerous corpora albicantia. The other fallopian tube measured 8.5 cm. in length. The distal third of its superior portion was transformed into a diffuse, globoid mass, 3 by 2 by 2.5 cm. in diameter (Fig. 1). The fimbriated end was patent and the portion of the tube 2.5 cm. distal to the mass was of normal appearance. On sectioning, the globoid mass was moderately firm and light gray, mottled with pale yellow-gray. Although the lumen of the fallopian tube appeared to end abruptly at the site of the tumor mass, the lesion appeared to be in the subserosa and muscularis and to compress the lumen. The tube proximal to the mass was patent.

Microscopic Description

Frozen sections revealed the tumor to be covered by interlacing bundles of smooth muscle fibers, widely separated by loose, edematous connective tissue which was infiltrated by focal accumulations of lymphocytes and scattered, pale-staining histiocytes with slightly vacuolated cytoplasm. These lymphocytic infiltrations were noted particularly about some of the thin-walled blood capillaries lying in the connective tissue stroma. No abnormality was seen in the serosa.

Within the muscular layer was a circumscribed tumor mass composed of numerous small acini, some of them lined by high cuboidal epithelium (Fig. 2). The cytoplasmic membranes of these cells were somewhat indistinct. The basilar nuclei were round or oval; the chromatin was pale-staining and vesicular. The lumina of these gland-like structures contained desquamated epithelium and an occasional lymphocyte (Fig. 2). The chromatin of desquamated epithelial cells was more compact than that of cells lining the acini.

The acini were surrounded by fine strands of connective tissue in which were embedded thin-walled capillaries lined by elongated endo-

thelial cells. Slight infiltrations of lymphocytes were noted in the delicate connective tissue stroma in the periphery of the tumor mass near the muscular layer.

In the central portion of the tumor mass there were distinct solid cords of cells with ample pink-staining cytoplasm (Fig. 3), and distinct cytoplasmic membranes. In some of the cells of the cords the cytoplasm was finely vacuolated. The structure of the nuclei was similar to that of the cells lining the acini. Seeming transition from cords of cells to acinar structures was demonstrable in the section. Within the tumor mass were scattered thick-walled blood vessels. Near the muscularis some of the acini appeared to extend into the stroma between the muscle fibers but did not go beyond the inner layer of smooth muscle tissue.

Fat stains revealed minute fat droplets in occasional histiocytes lying in the stroma of the acini near the muscular layer. A larger number of fat-laden histiocytes were lying among the infiltrating nests of lymphocytes within the muscle wall. The vacuolated spaces in the cells of the cords were free of fat.

Paraffin sections of this tumor mass from approximately the same area, when stained with hemalum and eosin, van Gieson, and Weigert's elastica stains, showed a markedly altered picture as compared with the frozen sections. In the hemalum and eosin stain the acini frequently were represented by acellular borders or by single flattened cells adherent to the wall of the acinus which contained two or more desquamated epithelium-like cells (Fig. 4). The cytoplasm of these cells was pink-staining and their nuclei were round and coarsely vesicular. In other places the acini were lined by flattened cells with indistinct cell membranes producing a syncytial effect (Fig. 4). In still other places the lining cells were high cuboidal. Where cords of cells were seen, the cytoplasm contained large vacuoles so that some of the cells assumed a signet ring appearance (Fig. 5). In other cells the cytoplasm was rather homogeneous bluish-pink staining. The nuclei of such cells were fairly large and somewhat irregular in size and shape. The chromatin was finely vesicular.

The muscle layer covering the tumor mass showed small focal infiltrations of round cells in the interstitial tissue, and appeared to surround the entire circumference of the tumor mass except for one portion which was attached to the mucous membrane of the fallopian tube. Van Gieson's stain revealed delicate connective tissue fibers between the acini and cords of cells. In places, single cells of the cords appeared to be isolated by fine, delicate, connective tissue fibers simulating the

interstitial epithelial cells described by Golden and Ash.³ The Weigert elastica stain showed distinct fibrils surrounding the acini and cords of epithelial cells.

Diagnoses. Tubular adenoma of a fallopian tube; multiple fibromyomata of the uterus.

CASE 2

S. C., a colored woman, 45 years old, gravida 0, para 0, was first admitted on August 13, 1939 (no. S-5706-39), with the history of having been seized with severe epigastric pain. She was admitted to Cook County Hospital as a surgical emergency. A diagnosis of incarcerated umbilical hernia was made and an operation was performed. At operation multiple fibroids of the uterus were noted and an inflammatory lesion of the right adnexa. The patient had a smooth post-operative course and was discharged on August 24, 1939, 11 days after operation.

Her second admission was on September 2, 1939, when she complained of pain low in the abdomen and radiating down the left leg. This pain had been present at intervals since her return home from the hospital. She was admitted mainly because of the large fibroids of the uterus which were felt on examination. At operation the uterus, right tube and ovary, and the appendix were removed. The postoperative course was uneventful.

Pathologic Report

The specimen consisted of a uterus amputated above the cervix, the right fallopian tube, and ovary. The uterus measured 20 by 18 by 11.5 cm. and was deformed by subserous, intramural, and submucous tumors, the largest measuring 8 cm. in diameter.

The fimbriated end of the fallopian tube was patent; the mucosa was light purplish gray and smooth. In the wall of the tube, near the serosal surface, was a nodule which measured 7 mm. in diameter and on section appeared light gray. It slightly compressed the lumen of the tube in its mid-portion; however, the lumen admitted the passage of a fine probe through the entire length of the tube.

The ovary measured 7.5 by 5.5 by 4 cm. and on sectioning contained a cystic corpus luteum 4 cm. in diameter.

Microscopic Description

Paraffin sections of the tumor nodule of the fallopian tube revealed it to lie in the deep layers of the muscle wall. In one area the tumor mass extended into the stroma of the folds of the mucous membrane and flattened out some of the folds. The stroma and the muscle bundles surrounding the tumor mass were infiltrated by a small number of lymphocytes, especially around some of the capillaries. The tubal folds were lined by tall-columnar, ciliated epithelium. The stroma was increased in amount and moderately vascular.

The tumor proper was composed of acini lined, in most instances, by flattened epithelial cells with indistinct cytoplasmic membranes

and somewhat elongated, flattened nuclei (Fig. 6). In other places these glands were lined by cuboidal epithelium with rather indistinct cytoplasmic membranes (Fig. 6), and vesicular nuclei. The lumina of many of these glands contained large desquamated cells with pale, pink-staining cytoplasm and small, deep-staining pyknotic nuclei. Between the acini were solid nests and cords of cells with rather distinct cytoplasmic membranes and pale-staining cytoplasm which was frequently vacuolated. The nuclei of these cells were oval and similar to the nuclei of the cells lining the gland structures, particularly those of the cuboidal epithelial type. The cytoplasm of the cells which composed the solid cords was similar in appearance to the cytoplasm of the desquamated cells lying within the lumina of the gland structures.

Van Gieson's staining of the tumor revealed small, delicate strands of connective tissue between some of the acini and cords of epithelial cells. Near the thick layer of muscle there were several glands lined by high-cuboidal epithelium showing distinct cytoplasmic membranes. Elastin-H staining revealed small, delicate elastic tissue fibrils around individual acini and cords of cells (Fig. 7). Here, as in case 1, individual cells of the cords might be surrounded by elastic fibers. In places the cords of cells seemed to split, forming small acini.

Diagnoses. Adenoma of a fallopian tube; cystic corpus luteum; multiple fibromyomata of the uterus.

CASE 3

C. L., a colored woman, 32 years of age, gravida 3, para 1, was admitted on January 21, 1942 (no. S-584-42), with complaints of pain in the right lower quadrant, vaginal discharge, fatigue, and dizziness. The patient stated that she had been well until January, 1940, when a vaginal discharge and "dropping of the womb" were noted. In August, 1941, she developed dizziness and fatigue and in January, 1942, she experienced dysmenorrhea, dysuria, and pain in the right lower quadrant. The pain was dull and frequently radiated to the epigastrium.

Abdominal examination revealed a hard, fixed mass in the supra-umbilical region which, on rectovaginal examination, was found to extend into the cul-de-sac. The adnexa seemed to be fixed to the major mass. A second degree prolapse of the uterus was present.

On February 2, 1942, a supracervical hysterectomy, bilateral salpingectomy, and right oophorectomy were performed. The postoperative course was uneventful.

Pathologic Report

The specimen consisted of a uterus amputated above the cervix, both fallopian tubes, and an ovary. The uterus measured 7.5 by 6.5 by 5.5 cm. and was deformed by subserous and intramural nodules which measured up to 12 mm. in diameter. The sectioned surface of these was grayish white and distinctly trabeculated. The fimbriated

ends of both fallopian tubes were occluded. Their walls were thickened and the mucosa was light gray. In the distal third of the right tube there was palpated a firm, round nodule which measured 1.5 by 1.5 by 1 cm.; the consistency was firm and the sectioned surface was grayish white. The ovary measured 6.5 by 4.5 by 4.5 cm. and contained a corpus luteum cyst, 4.5 cm. in diameter.

Microscopic Description

Section of the tumor mass in the fallopian tube revealed it to lie in the deep layer of the muscle and to encroach upon the lumen. The latter showed simple, fine, delicate folds, some of which were flattened by the proliferating tumor mass. The tumor was composed of numerous acini, many of them lined by flattened cells with elongated nuclei and indistinct cell membranes. In other places these acini were lined by cuboidal epithelium with coarsely vesicular nuclei (Fig. 8). The lumina of many of these glands contained desquamated epithelial cells with pink-staining cytoplasm and rather indistinct cytoplasmic membranes. In places small cords of cells could be seen with rather compact, coarsely vesicular nuclei, resembling the cells lining some of the acini. In the periphery of the tumor near the inner layer of muscle were small focal accumulations of lymphocytes.

Diagnoses. Adenoma of a fallopian tube; corpus luteum cyst; multiple fibromyomata of the uterus.

In summary, these three adenomas of the fallopian tube varied in size from 7 mm. to 3 by 2 by 2.5 cm. The tumors were rather firm and the cut surface was diffusely grayish white and smooth. In all three instances the tumor seemed to have originated in the muscle wall and extended toward the mucous membrane, in places flattening the folds. All tumors were globular and grossly circumscribed. In case 1, suggestive invasion of the adjacent muscle tissue was noted in one small area; however, this was the only area in which such a picture was seen and may not necessarily represent true invasion of the muscle wall.

The microscopic picture of the three tumors presented here was rather uniform in so far as it revealed large and small acini lined by flattened or cuboidal epithelial cells. These glands were separated by fine, delicate strands of vascular fibrous connective tissue. The lumina of some of the glands were filled with desquamated epithelium-like cells similar to those found lining the small and large acini. Red blood cells and amorphous staining substances were not noted. Smooth muscle tissue within the tumors, as described by Golden and Ash,³ was

not noted in our series of cases. Weigert's elastica stain and elastin-H showed fine elastic fibers surrounding the acini as well as cords of epithelial cells. A fat stain on case 1 showed fat deposits in small groups of histiocytes at the periphery of the tumor mass. However, no fat was observed in the acinar cells or in the cords of the tumor cells. Unfortunately, the histologic picture was not anticipated in cases 2 and 3 and frozen sections were not made. Solid cords of cells were noted in all three cases, particularly in case 1, which was prepared by the frozen tissue method. Many of the cells in the cords contained vacuoles of variable size; others had a finely granular eosinophilic cytoplasm. Scattered lymphocytes were noted in the interstitial tissue of the tumor mass, particularly at the periphery.

DISCUSSION

It is apparent, from a review of the literature, that great discrepancy exists among the expressed opinions as to the pathogenesis, cytogenesis, and significance of the tumor described here. These tumors have been considered malignant by some investigators^{4,5} whereas others have called them benign.⁶⁻⁹ Evans^{1,2} was of the opinion that these tumors are mesothelial in origin since in one of his four cases he was able to demonstrate direct continuity of cells forming the serosa of the uterus with cells lining the gland-like structures of the underlying tumor. He also pointed out that although gland-like structures were present, the cells did not resemble true glandular epithelium, and that they were flat cells with a tendency to chain formation, the latter being typical of mesothelial cell structure. Golden and Ash,³ on the other hand, were unable to demonstrate direct continuity between serosal cells and tumor cells. In our three cases both the gross and the microscopic appearances pointed to a mid-wall origin with proliferation toward the serosa and mucosa of the fallopian tube. In no instance were we able to demonstrate continuity with the serosal surface of the tube. As a matter of fact, the encircling muscle bundles were thicker at the serosal aspect of the tumor than at the mucosal aspect.

The information which we consider most important as to the nature of the tumor mass was derived from the frozen sections. In these, it was apparent that the acini are lined by cuboidal epithelium and that the cords of cells are distinctly epithelial. A section of the same tumor prepared by paraffin impregnation was sufficiently altered to make a differentiation between epithelium-lined and mesothelium-lined spaces difficult.

Muscle fibers were not noted within the content of the tumor, but

where they were present, near the periphery of the tumor, they appeared to be pre-existent, as pointed out by Golden and Ash.³

From our studies we cannot venture a definite opinion as to the genesis of this tumor cell. Golden and Ash,³ in their studies of frozen sections from testes, epididymides, and their adjacent structures from stillborn infants and fetuses, were unable to demonstrate cell structures of the type seen in the tumor. However, they leave the question open in view of the fact that the method of sampling employed was inadequate.

We are of the opinion that these tumors are of epithelial origin; this opinion is supported by findings in frozen sections and elastic tissue stains, the latter showing elastic fibrils about individual acini and cords of cells. At no time were we able to demonstrate any similarity between cells lining the blood vessels and cells lining the acini. The cells of the acini which we encountered, although frequently flattened, were different in that they lacked sharp tapering ends and did not have a spindly appearance. Furthermore, one would expect associated inflammatory changes to coexist with a serosal cell proliferation, giving rise to mesothelial cells resembling epithelial cells. In our series, inflammatory changes were by far too insignificant to account for tumor growth of such magnitude.

The diagnosis of adenoma for this type of tumor has been suggested by Gordon-Taylor and Ormman-Davis,⁷ and Blumer and Edwards.⁸ Golden and Ash,³ on the other hand, have suggested the term adenomatoid tumors of the genital tract which perhaps more fully fits the morphologic picture, in view of its questionable genetic origin.

SUMMARY

Three cases of epithelial tumors of the fallopian tube are reported. The clinical picture and the gross and histologic findings point to a benign epithelial tumor of obscure origin. The term "adenomatoid tumor" is viewed with favor to designate this tumor.

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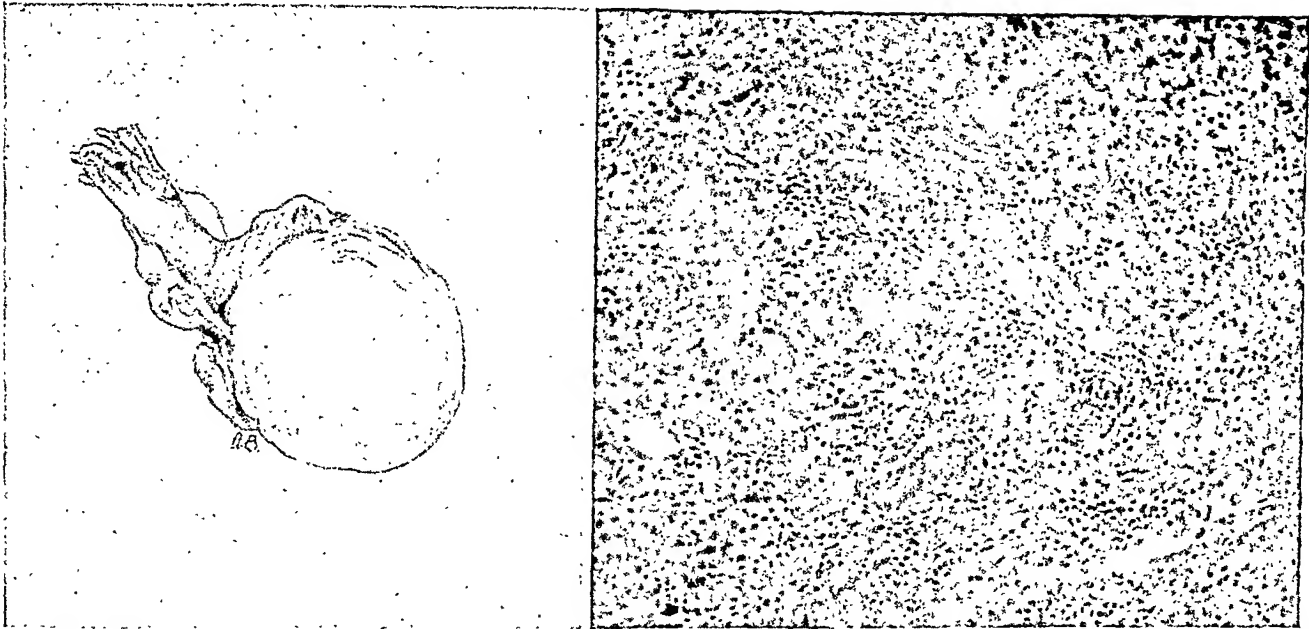
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DESCRIPTION OF PLATES

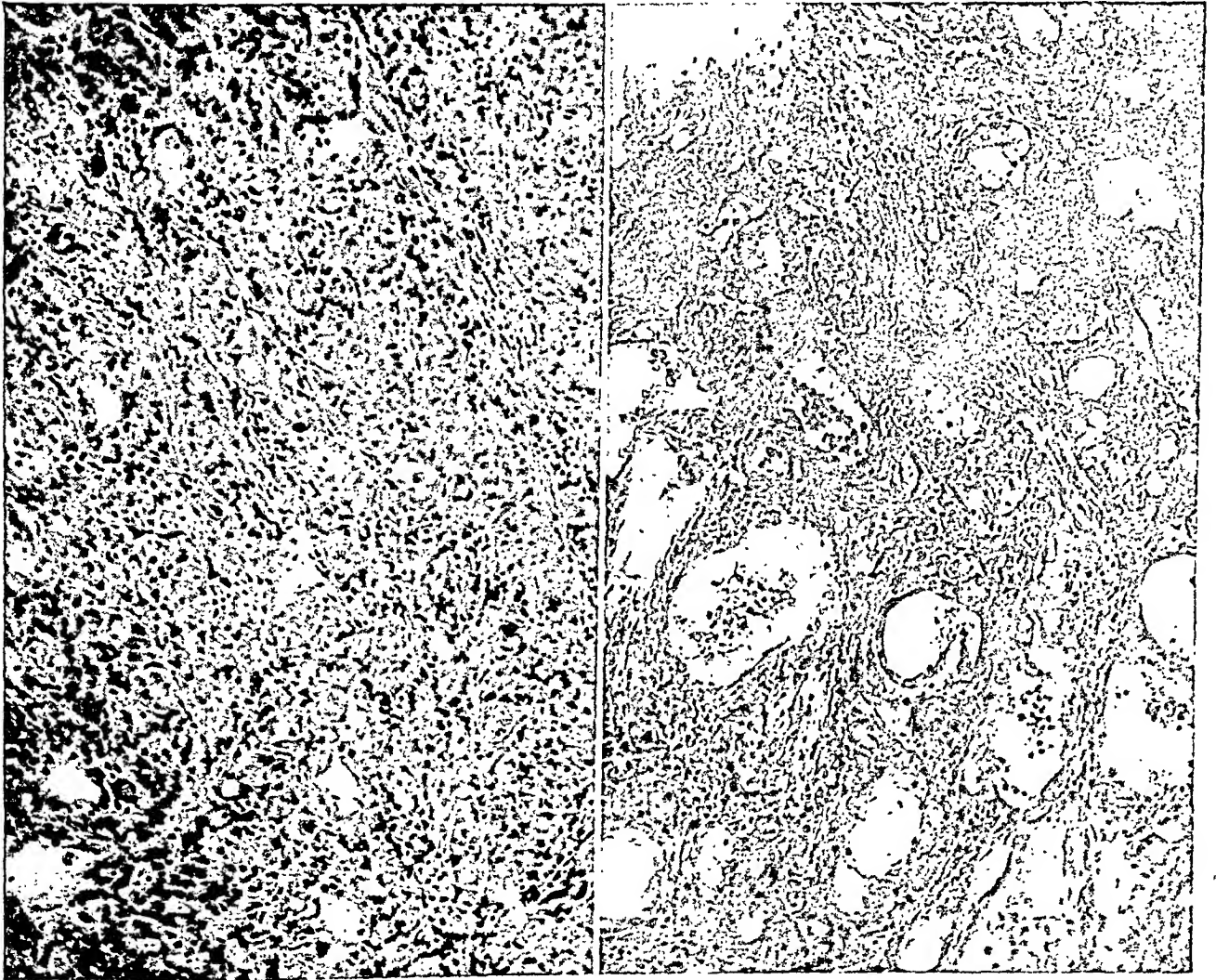
PLATE 153

- FIG. 1. Case 1. Drawing of specimen removed at operation showing fallopian tube and the tumor mass.
- FIG. 2. Case 1. Acini are lined by cuboidal epithelium, and desquamated epithelial cells lie within the lumen of the acini. Frozen section; hemalum and eosin stain. $\times 160$.
- FIG. 3. Case 1. Solid cords of cells of epithelial character. Frozen section; hemalum and eosin stain. $\times 160$.
- FIG. 4. Case 1. Acini with acellular borders, lumina containing desquamated epithelial cells. There are also acini lined by flattened cells with indistinct cell membranes producing a syncytium-like effect. Paraffin section; hemalum and eosin stain. $\times 160$.

1



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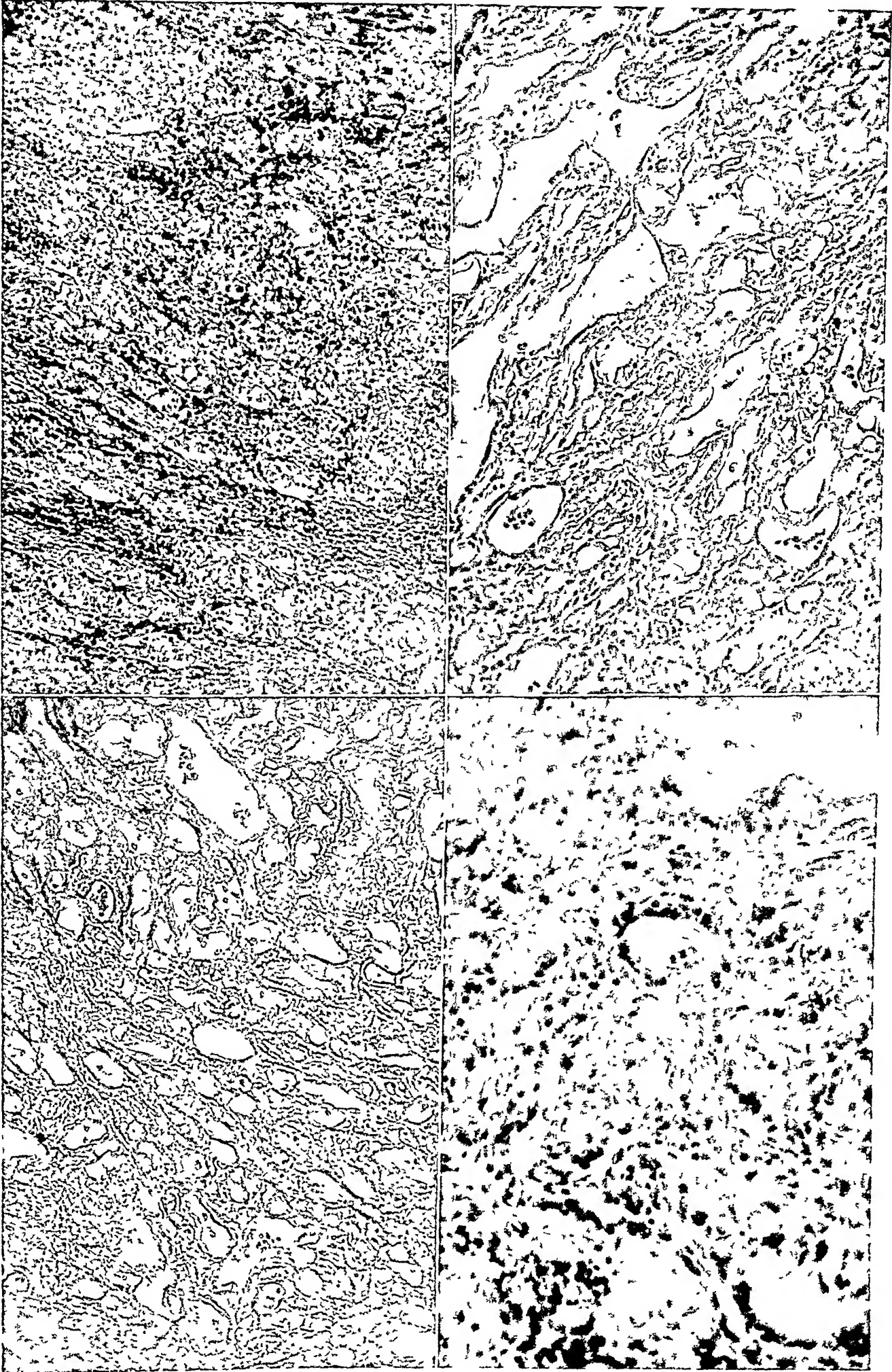


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PLATE 154

- FIG. 5. Case 1. Solid cords of cells with vacuolization of the cytoplasm. Paraffin section; hemalum and eosin stain. $\times 160$.
- FIG. 6. Case 2. Desquamated epithelial cells are seen in the lumen of acini lined by flattened epithelial cells; also acini lined by cuboidal epithelium. Paraffin section; hemalum and eosin stain. $\times 160$.
- FIG. 7. Case 2. Fine elastic tissue fibrils are seen about acini and cords of cells. Paraffin section; elastin-H stain. $\times 160$.
- FIG. 8. Case 3. Acini lined by cuboidal epithelium. Paraffin section; hemalum and eosin stain. $\times 320$.



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GASTRIC CANCER: MORPHOLOGIC FACTORS IN FIVE-YEAR SURVIVAL AFTER GASTRECTOMY *

PAUL E. STEINER, M.D., SAMUEL N. MAIMON, M.D., WALTER L. PALMER, M.D.,
and JOSEPH B. KIRSNER, M.D.

*(From the Department of Pathology, and the Frank Billings Medical Clinic of the
Department of Medicine, The University of Chicago, Chicago 37, Ill.)*

The prognosis in gastric cancer is generally recognized as poor. Surgical resection offers the best hope of eradication. It has been stated that the over-all cures are less than 5 per cent,¹⁻³ although in select groups subjected to operation the rate is higher.⁴⁻¹⁰ Following recovery from resection, any of the following events with respect to the cancer itself may occur: (a) Fatal termination within 5 years from local recurrence or distant metastases by tumor which was not evident at the time of operation; (b) five-year survival, but subsequent reappearance of the tumor; (c) permanent cure, with no residual cancer, overt or occult.

These old observations raise many questions, such as the following: (1) Is it possible to distinguish between group *a* on the one hand and groups *b* and *c* on the other—between those who will succumb quickly to recurrence or metastases and 5-year survivors? In other words is it possible to forecast outcome? Can factors with prognostic significance be found? (2) What is the explanation for the slow rate of growth of some gastric cancers so that more than 5 years are required for the formation of recurrences or metastases of clinical significance from residual microscopic tumor foci? (3) In 5-year survivors what are the relative proportions of truly cured cases to those with delayed reappearance of residual tumor?

Having at our disposal suitable material, we have sought answers to some of the questions. The material consists of 30 cases in which the patients lived 5 years or longer after resection of a gastric cancer. All available clinical records and gross and microscopic material were

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reviewed. In addition to hematoxylin and eosin stains, the chrome hematoxylin-phloxine and silver impregnation (for argentaffine quality) methods of Gomori were used for cytoplasmic details, and a modified Wilder's silver impregnation was employed to study the finer fibrillar material. Special stains for mucus were made as needed.

It was anticipated that some cases might have to be eliminated from the group because they were not malignant tumors or perhaps even not true neoplasms. This was found to be unnecessary. All cases conformed to the standard criteria for malignant tumors; none were borderline. All exhibited neoplasia, anaplasia, and heterotopia, infiltrating through the muscularis mucosae and, with one exception, into or through the true muscularis.

Study of the 30 5-year survivors revealed some microscopic features which were not expected from our previous experience with gastric cancer. Therefore, a special control group was used for specific and detailed comparison. It was composed of 30 cases of carcinoma of the stomach in which the patients had undergone resections similar to those of the 5-year survivors, had survived operation, but had died of local recurrence or metastases within 1 year after operation. (Hereafter these are designated "short-term" survivors.) Many of them had died within 6 months. Thus, the extreme ends of the growth-rate spectrum were under comparison. It was hoped that this would reveal, to a maximum degree, any differences associated with rate of growth and degree of malignancy.

For a few comparisons with the 5-year survivors, the entire clinical group of 576 cases of gastric cancer was used.¹¹

Attempts to find valid criteria for estimating prognosis have been made previously. One of the earliest studies was that of Whipple and Raiford⁵ who combined gross and microscopic grading systems and found them useful. Broders¹² made a histologic grading of a large series of cases. The 5-year survival rates were 86.2, 58.8, 30.2, and 23.3 per cent, respectively, in grades 1, 2, 3, and 4, so that there was fair correlation with group prognosis. Dochat and Gray¹³ attempted to estimate prognosis by a combination of Dukes' macroscopic classification¹⁴ (originally developed for rectal cancer) and Broders' microscopic grading method.¹⁵ They found the combination better than either alone. Eker¹⁶ also used a combination of gross and microscopic grading and found some, although imperfect, correlation between the results of grading and prognosis. Schindler, Steiner, Smith, and Dailley¹⁷ found little of prognostic value in microscopic grading but greater

value in macroscopic typing. In a later study, Schindler¹⁸ carried macroscopic typing further and observed that the tumors in all 5-year survivors were of the noninfiltrating types I and II. Stout¹⁹ expressed the opinion that histologic grading, based on cell differentiation, was of little value in predicting degree of malignancy, rate of growth, or probable success or failure of surgical treatment.

The consensus seems to be that either macroscopic typing or microscopic grading alone are useful, that combined they are better, but that they can be used only for group and not for individual case prognosis because of frequent discrepancies. In all large series of cases a few patients who should be alive are dead, and others with a supposedly bad prognosis are alive. It was hoped that new and more reliable factors might be revealed by the present analysis.

PROGNOSTIC CRITERIA

INFORMATION FROM CLINICAL STUDIES

In earlier studies by some of the members of our group^{11,20,21} no reliable clinical prognostic criteria were found. On the contrary, some unexpected and disturbing observations were made. For example, it was noted that short duration of symptoms before operation, however important this might be in selected cases, was of no prognostic significance in this group of 30 5-year survivors. In fact, long duration was more common in the 5-year survivors than in the large group as a whole, exceeding 1 year in 50 per cent of the former (nearly 20 per cent had symptoms for 2 years) in contrast to 30 per cent of the latter. Presence or absence of a palpable tumor also was of no prognostic importance; it was present in 9 of the 5-year survivors as compared with 16 of those who survived less than 1 year. Hemoglobin values, hydrochloric acid content of the gastric secretion, and presence or absence of occult blood in the stools likewise had no prognostic significance.

A comparison of the sex ratios in the 5-year and short-term survivors revealed 18 males (60 per cent) and 12 females (40 per cent) in the former, and 26 males (87 per cent) and 4 females (13 per cent) in the latter group. The corresponding figures for all of our gastric cancers, 576 patients, are 69 per cent males to 31 per cent females,¹¹ a ratio generally unexplained but like that reported by others. The group of 5-year survivors had a higher proportion of females and the short-term survivors more males than did the entire group. This difference in the prognosis in relation to the sexes remains unexplained.

It corresponds to that in some other cancers, notably carcinoma of the lung,²² and it may be related to a degenerative change in tumor cells, to be described.

INFORMATION FROM GROSS FEATURES

Location and size of the tumors were of no value in estimating prognosis; they were about the same in the long- and short-term survivors. The exception to this statement was that the latter group included several examples of diffuse, infiltrating carcinoma in which the extent and hence the size of the tumor were not determinable grossly. The largest tumor associated with 5-year survival was 10 by 9 by 6.5 cm.; with less than 1-year survival the largest was 15 by 14 by

TABLE I
*Comparison of the Macroscopic Type of Tumor in Five-Year
and Short-Term Survivors*

	Macroscopic types			
	I	II	III	IV
Five-year survivors	9	17	4	0
Survival less than 1 year	1	12	7	10

8 cm. (843 gm.). The average diameter of the tumor was about 6 cm. in each group.

The only significant prognostic gross factor was the macroscopic typing based on the appearance of the excised specimen. The standards used in gross typing were those previously published,¹⁷ so that it is unnecessary to repeat them. They resemble those advocated by Borrmann.²³ Four types were used, as follows: type I, polypoid carcinoma; type II, noninfiltrating carcinomatous ulcer; type III, infiltrating carcinomatous ulcer; type IV, diffuse infiltrating carcinoma. Those cases in which the cancer was well delimited by distinct raised margins had the best prognosis, but the correlation was not perfect.

✕ The results of the macroscopic comparison of the two groups are given in Table I. All persons except one with a type I tumor survived for 5 years and all with type IV tumor were dead within 1 year, but between these extremes lie two-thirds of the cases with overlapping in type and prognosis. In these, typing was of no prognostic aid. No doubt if cases with intermediate length of survival, namely, 2, 3, and 4 years, were tabulated, the correlation with types I and IV would be less striking. Macroscopic typing used alone would have given a bad prognosis to 4 (types III and IV) of the 5-year survivors, and a good

prognosis to 13 (types I and II) of the short-term group, introducing such a large error as to make the method of little prognostic value.

INFORMATION FROM MICROSCOPIC FEATURES

Three microscopic features were found which were correlated with long survival. In the order of their importance they were: Sharp circumscription of the tumor; two special histologic types; and a characteristic retrogressive change in tumor cells. They will be described in that order, and followed by several other microscopic findings of lesser importance.

Circumscribed Growth

Twenty-five tumors in the 5-year survivors grew in a circumscribed manner, exhibiting a distinct demarcation between tumor and host. The margin of the tumor was usually smooth but sometimes it was scalloped. The advancing border of the tumor was sometimes at the true muscularis, sometimes deep in the muscle layers, and sometimes in the serosa, but it was always fairly sharp, and distinct. *The tumor advanced through the gastric wall en bloc.* This feature is illustrated in Figures 1 to 4. With the lowest magnification this demarcation appeared sharp; with higher magnification a slight amount of intermingling and interdigitation of tumor cells and normal cells was observed occasionally. These cancers, contrary to the implications of their designation, had no crab-like extensions or "roots." The circumscription was not due to a fibrous capsule, which in many cases was incomplete, imperfect, or absent (Figs. 7 to 9). Neither was it due to an unusual degree of mutual cohesiveness of the tumor cells (Fig. 15).

In the 30 cases with survival of less than 1 year, a similar degree of circumscription was found only twice; the remainder were noncircumscribed (Figs. 5 and 6).

Histologic Types

Most of the histologic types of gastric cancer were represented in the group of 5-year survivors, as is shown in Table II, and in Figures 10, and 13 to 16. At first glance there seemed to be no relation of histologic type to prognosis. On closer analysis, however, and by comparison with the short-term survivors, two types of cancer (representing 9 cases) were found only in the 5-year group. They carried a favorable prognosis. They are a special type of undifferentiated carcinoma, to be described, and the well known undifferentiated small cell carcinoma (or sarcoma).

Six undifferentiated carcinomas of a special type formed the largest histologic group associated exclusively with good prognoses (Figs. 7, 8, and 14). These tumors were composed of groups and solid cords of medium-sized polyhedral cells lying in a cell-rich stroma, which comprised about one-half of the area. The cells had large, pale, often irregular nuclei and little cytoplasm. The stroma was rich in cells of the types associated with chronic inflammation. For this reason and because of the basophilic nature of the cancer cells themselves, these tumors had a distinctly bluish appearance under the lower magnifications when stained with hematoxylin and eosin. We have referred to them among ourselves as "the blue cell cancers." These tumors had no products of maturation or function, or other morphologic features by which their histogenetic nature could be identified. With special stains they had no argentaffine granules. With the idea that they might be chief cell carcinomas, they were stained with the chrome hematoxylin-phloxine stain, but they contained no cytoplasmic granules. The

TABLE II

Comparison Between Histologic Classification and Macroscopic Type in Five-Year and Short-Term Survivors after Gastrectomy for Cancer

Histologic type	Five-year survivors, macroscopic type					Surviving less than 1 year, macroscopic type				
	I	II	III	IV	Total cases	I	II	III	IV	Total cases
Adenocarcinoma	7	6	I		14		8	3	5	16
Special undifferentiated carcinoma	I	4	I		6					
Undifferentiated small round cell tumor		3			3					
Mucinous carcinoma		I	2		3	I	I		I	3
Mixed type carcinoma	I	2			3					
Infiltrating scirrhous carcinoma		I			I		3	4	4	11
Totals	9	17	4		30	I	12	7	10	30

cytoplasm had a slight diffuse affinity for the phloxine. The best clues regarding their nature were supplied by 2 cases in which growth of this type was mixed with adenocarcinoma and intermediate forms, and by a third case in which there was mixture with colloid carcinoma. Because they appeared dark and undifferentiated they might, *a priori*, erroneously be regarded as highly malignant. All were sharply circumscribed. One had extensive metastases to regional lymph nodes. Five of these tumors were found in men. There were no tumors of this type in the short-term survivors. This carcinoma should be set apart in classifying gastric cancers. In this series of cases it was always associated with a good prognosis, despite the fact that histologically it would be grade 4.

Three cases in the 5-year survival group were classified as round cell

tumors. They might be reticulum cell sarcomas, although it is not certain that they are not highly undifferentiated medullary carcinomas (Fig. 15). They were circumscribed but nonencapsulated, with tumor cells in direct relation to muscle. The tumors were composed of small to medium-sized round to polyhedral cells growing compactly with little stroma. There was no follicle formation. Silver impregnation for reticulum, however, revealed an abundant network of fibrils which at some point or other made contact with nearly every cell. The patients survived for 5 years despite the fact that metastases were demonstrated in lymph nodes in 2 cases. There were no similar tumors among the short-term survivors. Histologically these tumors also would fall into grade 4, yet the prognosis was good.

Adenocarcinomas formed the largest histologic group in the 5-year survivors, with 14 cases. They presented no unusual features except possibly a remarkably slight secretion of mucus (Figs. 9, 12, and 13). In size the glands varied from small to giant, and in some cases they were mixed. The glands were round, cylindrical, or irregular, and some had papillary ingrowths. The amount of stroma varied from slight to considerable. According to the system of histologic grading previously described,¹⁷ based on the degree of cell differentiation and of deviation from normal appearance of the glands, 5 cases were grade 2, 7 were grade 3, and 2 were grade 4. Twelve of the 14 adenocarcinomas were circumscribed and 2 were infiltrative. The short-term survivors had 16 adenocarcinomas, all of which were noncircumscribed. Adenocarcinomas were associated with favorable prognosis only when they were circumscribed, and not always then.

There were three carcinomas with conspicuous secretion of mucus in each group. One infiltrative scirrhus carcinoma and 3 of mixed type completed the series of long-term survivors. The short-term survivors included 11 examples of infiltrative scirrhus carcinoma, some of which were of the linitis plastica type. Some infiltrated widely like linitis plastica but had in addition a local mass.

Retrogressive Changes

The chief retrogressive change was of a distinctive type. It consisted of atrophy and distortion of tumor cells and pyknosis of their nuclei. This change was most conspicuous at the advancing border of the tumor; less commonly it was seen throughout (Figs. 8 to 11). It was sometimes more striking at the advancing end of a gland than at the proximal end of the same gland (Fig. 12). It appeared to be independent of the character of the stroma. This retrogressive change was most impressive in an undifferentiated, noncircumscribed, scirrhus

carcinoma. This slightly ulcerated, flat, antral tumor measured 4.5 by 4.0 cm. From it there was widespread infiltration of the muscle layers and serosa by scirrhous tumor of linitis plastica type. Everywhere these cells showed marked atrophy and pyknosis (Fig. 10), resembling the histologic picture seen in prostatic carcinomas following orchiectomy. This patient was a female. Altogether, the same change was seen in greater or lesser degree in 14 patients, of which 8 were males.

The common types of degenerative change, such as massive necrosis, were less frequent in the long-term group than in the controls. This may be attributed to their slower rate of growth with better blood supply.

Metastases

Metastases were seen in lymph nodes in 6 of the 18 5-year survivors (33 per cent) in which material was available for study. The 6 cases consisted of 2 each of adenocarcinomas and round cell tumor, and one each of special undifferentiated carcinoma and mixed cell carcinoma. The corresponding figures for the short-term group are 17 cases with metastases in a total of 21 examined (81 per cent). Four in each group also showed infiltration into perigastric adipose tissue. Lymph node metastases cannot be used as an index of prognosis in individual cases although there is some difference in group prognosis. Neither should the presence of regional lymph node metastases *per se* obviate attempts at surgical cure.

Miscellaneous Histologic Features

The tumors of the 5-year survivors contained on the average more inflammatory infiltrate in their stroma than did those of the short-term survivors. This was most conspicuous in the special "blue cell" cancers already described. It was not consistent enough to be used in prognosis. The same may be stated with respect to the amount of dense connective tissue stroma and of tumor necrosis which were usually greater in amount in the short-term survivors. The number of mitotic figures was not discernibly related to prognosis, although counts were not made.

CURE VERSUS SLOW GROWTH AS EXPLANATION FOR FIVE-YEAR SURVIVAL

The question whether 5-year survival was due to cure or to slow rate of growth of the tumor cannot be answered as yet from this group of cases because 27 of the patients were alive when last traced. Two of those still living have had recurrences later than 5 years after oper-

ation. In the remaining 25 it is impossible to state how many are cancer-free and will never have a recurrence. One of these patients has, in the meantime, had treatment for two cancers of the face. Of the 3 no longer alive, 2 died of recurrent carcinoma at 22 and 6 years, respectively, after their first operation. The third patient died 7 years after operation, probably from a recurrence.

Thus, while it is impossible to state how many are cancer-free and cured, or how many have slow-growing, subclinical cancer residues, it is known that in 4 (and probably in 5) instances 5-year survival was made possible because the rate of tumor growth was very slow. Curiously, perhaps significantly, all 5 cases had adenocarcinomas.

CAUSE FOR SLOW RATE OF GROWTH

The cause of the slow rate of growth, whether low aggressiveness of the cancer cells, or high resistance of the host, or a balance between the two, remains unknown. Certain features seen in the 5-year survivors and absent to a comparable degree in the short-term survivors may, however, be the morphologic concomitants of the basic causes and worthy of record.

The most striking change was the atrophy and pyknosis of cancer cells previously mentioned and illustrated in several photomicrographs, notably in Figure 10. The remarkable resemblance to regressing cells in prostatic carcinoma following orchiectomy has been mentioned. It appeared to be primary, independent of the condition of the stroma, and not accompanied by inflammatory reaction. In one widely infiltrating scirrhous carcinoma it was seen in many parts of the growth; in circumscribed tumors it was most striking near the margin. This change involved only tumor cells, the adjacent stromal cells remaining normal in appearance, so that it is probably not on a vascular or nonspecific nutritional basis.

The sharp circumscription of the tumors could, theoretically, be attributed either to low aggressiveness of the tumor or to resistance on the part of the host. Regardless of whether it is the cause or effect of slow growth, it is useful in estimating prognosis.

All 5-year survivors had either a sharply circumscribed neoplasm, or severe retrogressive changes in the tumor cells, or both.

Although the group of 5-year survivors contained a higher proportion of females than did the entire group of 576 cases or the short-term survivors, the 14 cases showing the special degenerative change have the same sex ratio as the entire 5-year group. Their state with respect to hormonal balance is, however, unknown.

HISTOLOGIC GRADING OF GASTRIC CANCER

Histologic grading, however useful it may be in other tumors,²⁴⁻²⁶ has not been very helpful in estimating prognosis in gastric cancer.^{5, 12, 13, 16, 17} There are many grade 1 cases with short survival and some grade 4 cases with unexpectedly long life. The grading systems have been based principally on cell differentiation and on the degree of resemblance to, or deviation from, the normal glandular pattern. The failure of that method is explained by the fact that some forms of cancer which must be given a "bad" grade are now known to be associated with a good prognosis.

TABLE III

Comparison of Histologic Type and Former Microscopic Grade in Five-Year and Short-Term Survivors after Gastrectomy for Cancer

Histologic type	Five-year survivors, microscopic grade					Surviving less than 1 year, microscopic grade				
	1	2	3	4	Total cases	1	2	3	4	Total cases
Adenocarcinoma		5	7	2	14			3	13	16
Special undifferentiated carcinoma				6	6					0
Undifferentiated small round cell tumor				3	3					0
Mixed type carcinoma				3	3					0
Mucinous carcinoma				3	3				3	3
Infiltrating scirrhous carcinoma				1	1				11	11
Total	0	5	7	18	30	0	0	3	27	30

Table III presents a comparison of histologic type and old microscopic grade in the 5-year and short-term survivors. The special undifferentiated carcinomas and the undifferentiated round cell tumors, now known to have a favorable prognosis, must, by the grading system formerly employed, be given the unfavorable grade 4. Since they constitute nearly one-third of the 5-year survivors, a great error is introduced by them alone. The mucinous, mixed type, and many of the adenocarcinomas were also grade 4, yet their proved course is now known to have been favorable. Altogether, 25 of the 30 5-year survivors were given the unfavorable grades 3 and 4. The grading system was too simple to be successful, and it must now be modified.

Histologic grading appears to work best in tumors derived from simple epithelia in which cells of only one basic type are present. Good examples are the tumors arising from stratified squamous epithelium, transitional epithelium, or simple columnar epithelium, as in the rectum. When more complex epithelia are concerned, such as those in the mammary gland, lungs, and stomach (where the surface mucus-

secreting cells, neck cells, chief cells, parietal cells, argentaffine cells, and sometimes Paneth cells are present), the morphologic potentialities are so complicated that a simple grading system is inadequate. The potentialities of chief and parietal cells in pure tumor form are not known, to our knowledge. It is not known whether they would grow solidly or have lumina. To be successful, any grading system applied to gastric cancer must take into account the complex nature of the epithelium, and the different types of tumor derivable therefrom.

The histologic grading systems which have been applied to gastric cancer have placed chief emphasis on the degree of perfection of the glands, which is a reflection of the differentiation of the individual cells. Some have taken into account also the extent of the tumor and the method of extension. The former was poorly correlated with long survival because 10 of 18 patients in whom microscopic material was available showed metastases to regional lymph nodes (6 cases), or extensions into the perigastric tissues (4 cases), yet they survived for 5 years. However, we have found that the method of extension is very important in and of itself. When it is combined with the other two factors, namely, the two special histologic types and the special degenerative change, an excellent correlation with 5-year survival is found.

CORRELATION OF GROSS TYPE, HISTOLOGIC GRADE, AND HISTOLOGIC TYPE

In Table II a comparison is made between histologic type (not grade) of gastric cancer and macroscopic type of growth. Except for the special undifferentiated carcinomas and the undifferentiated small round cell tumors, both of which were of localized types (I and II), there is little correlation. Adenocarcinomas and mucinous carcinomas may be of any gross type from I to IV. Scirrhus carcinomas are usually of the infiltrative types III and IV.

In Table III a comparison is made between histologic type and microscopic grade. The adenocarcinomas were distributed among microscopic grades 2, 3, and 4. All other types were placed in grade 4 because the cells appeared highly undifferentiated. The lack of close correlation between histologic grade and clinical outcome has previously been stressed.

A comparison between microscopic grade and macroscopic type is given in Table IV. The latter shows a better correlation with outcome than does the former. There is some slight degree of correlation between them. That it is not greater is probably due to the imperfections in the microscopic grading system which was used.

COMMENT

This study revealed some factors associated with 5-year survival after gastrectomy which may be used in predicting prognosis. That the correlation was less than perfect may be attributed to the fact that the cancers in 5-year survivors do not constitute a biologically homogeneous group. Long survival may be due either to cure because of the fortuitous circumstance of complete removal of tumor cells, or to slow growth of residual cancer cells. The latter group is biologically homogeneous with respect to degree of malignancy but the former is not, being composed of two possible subgroups. Some may have been cured because, although highly malignant, the cancer cells were all removed; others were, no doubt, of low malignancy but were cured for the same

TABLE IV

Comparison Between Microscopic Grade and Macroscopic Type in Five-Year and Short-Term Survivors after Gastrectomy for Cancer

Macroscopic types	Five-year survivors, microscopic grades					Surviving less than 1 year, microscopic grades				
	1	2	3	4	Total	1	2	3	4	Total
Type I		3	4	2	9				1	1
Type II		1	3	13	17			1	11	12
Type III		1		3	4			1	6	7
Type IV								1	9	10
Total	0	5	7	18	30	0	0	3	27	30

reason. Inherently, the cells in some of the cases of true cure may be highly malignant and in others of low malignancy, but precise information as to which cases belong in each group is lacking because of absence of a biologic test, the tumors having been removed. The morphologic equivalents to high or low malignancy cannot be accurately determined by a study of this group. Theoretically, the highly malignant tumors should resemble morphologically those in the group of short-term survivors; the presence of such tumors in the 5-year survivor group would presumably be attributable to the removal of all cancer cells. Thus, possibly an error is introduced into any analysis of this group based on morphology. The cancers which recurred after 5 years form a more uniform biologic group in which the common factor is a demonstrated slow rate of growth. At the present time the 30 cases cannot be subdivided into these two biologic groups because the ultimate course is known in only 5. Some years hence, when accurate subclassification becomes possible, it may be possible to establish still more accurate relationships between morphology and prognosis.

The cancers in the group surviving less than 1 year, unlike the 5-year

survivors, constitute a homogeneous biologic group with respect to rate of growth. Inasmuch as they grew from microscopic residues to produce fatal masses within a period of 1 year, they were uniform in having a rapid rate of growth. This homogeneity had its morphologic equivalents in noncircumscribed growth, absence of two favorable histologic types of tumor from the group, and absence of the special retrogressive change in its fully developed form.

It should be emphasized that study of the margins of a gastric cancer in microscopic sections properly prepared is more precise than is gross inspection. The numerous attempts in the past at macroscopic typing have been consistent in demonstrating considerable merit in the method, which often uses sharpness and elevation of the tumor margin as one criterion. However, the microscopic extent of a lesion is frequently greater than is visible to the unaided eye. Eker¹⁶ has seen microscopic extensions 5 cm. beyond the apparent margin of the tumor, and this observation can be confirmed by many others. For this reason degree of circumscription of a tumor as determined microscopically should be more reliable than gross typing.

From the 5-year survivors and the controls it is possible to determine the amount and cause of the error in prognosis by macroscopic typing, histologic grading, or a combination. Four of the 30 5-year survivors would have been given an unfavorable prognosis by macroscopic typing, being of type III (Table II), and 13 of the short-term group would have been given a good prognosis, inasmuch as they were of types I and II (Table II). Twenty-five of the 5-year survivors would have been given a bad prognosis because they were in microscopic grades 3 and 4 (Table II). Only 4 cases would have been given a good prognosis by a combination of microscopic grades 1 and 2 and macroscopic types I and II (Table IV). The error obviously would have been large by any of these methods. Macroscopic typing, alone, would have been better than the combination.

It may be worthy of note that the method used to find factors which have prognostic significance was different in this than in previous studies. It has been customary to apply predetermined criteria or standards to a series of treated cases and to observe the correlations with length of survival. In the present investigation cases with known end-results were analyzed to discover factors not present in a control group of short-term survivors.

SUMMARY

Thirty cases of gastric cancer in which the patients had survived for 5 or more years after resection were compared with a control group of 30 patients who had died of recurrence within 1 year. Efforts were

made to find factors with prognostic significance, explanations for the slow rate of growth observed occasionally, the relative importance of cure versus delayed reappearance of residual tumor cells as an explanation for 5-year survival, and defects in grading systems for gastric cancer.

Factors associated with 5-year survival were sharp circumscription of the tumor, two special histologic types of cancer, and a characteristic degeneration of tumor cells. These factors were more reliable in predicting outcome than were histologic grading or macroscopic typing or a combination of these methods. While the morphologic concomitants of slow rate of growth have been described, the causes remain unknown.

Five-year survival after gastrectomy for cancer may be due to cure by complete extirpation or to slow rate of recurrence. The relative importance of these two factors cannot be determined until the ultimate outcome is known.

The chief defect in macroscopic typing as a method for estimating prognosis lies in its inability to detect the finer degrees of circumscription of the tumor. The chief defects in histologic grading are the failure to recognize the favorable course of a few special types of undifferentiated cancer, and the importance of circumscription and retrogressive changes in the tumor.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 155

Low-power photomicrographs ($\times 3$) to compare circumscribed growth, irrespective of histologic type, in 5-year survivors (Figs. 1, 2, 3, and 4) with infiltrative growth in short-term survivors (Figs. 5 and 6).

FIG. 1. An undifferentiated cell carcinoma which has penetrated through the gastric wall *en bloc*. Figure 7 is a higher magnification showing the details of this tumor and of the junction of tumor and host.

FIG. 2. An adenocarcinoma with similar deep penetration *en bloc* into muscle.

FIG. 3. A circumscribed colloid carcinoma which is growing into muscle. Figure 16 is a higher magnification of this tumor.

FIG. 4. This is an undifferentiated round cell tumor, similar to that shown in Figure 15, which has penetrated through the true muscle layers in circumscribed but nonencapsulated form.

FIG. 5. This is an undifferentiated adenocarcinoma which is not circumscribed. Tumor is seen in the serosa and the perigastric adipose tissue. It is separated from the main tumor by intact muscularis.

FIG. 6. A noncircumscribed mucinous carcinoma with infiltration into the serosa, by-passing intact muscle.

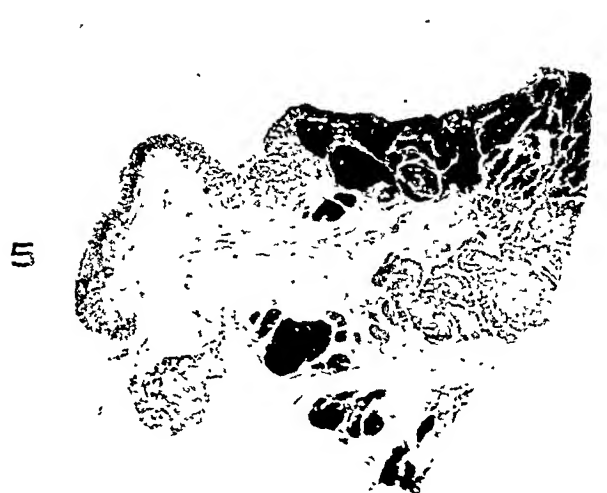


PLATE 156

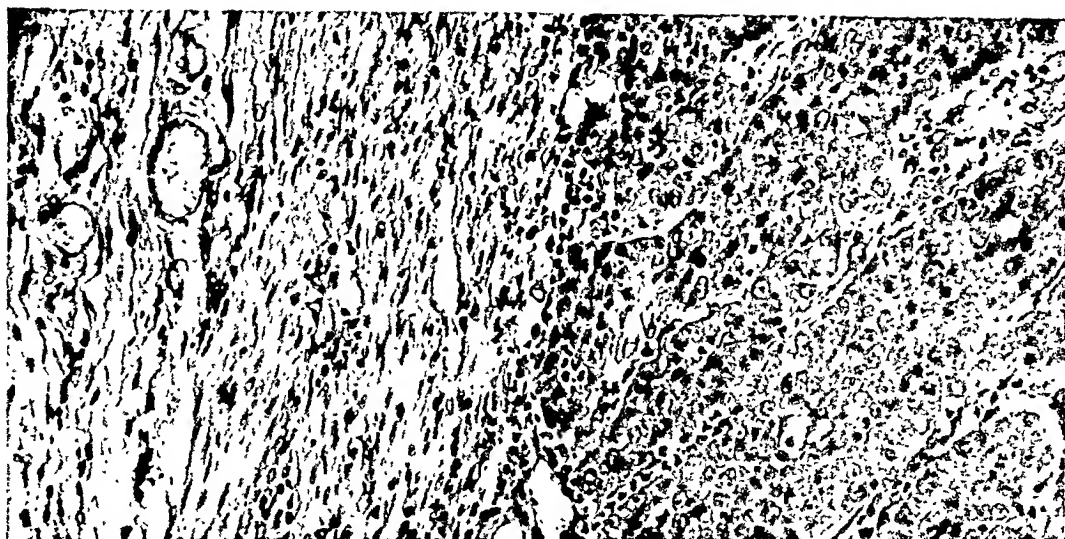
Three types of histologic reaction ($\times 200$) at the margins of three tumors of two different histologic types.

FIG. 7. Special undifferentiated carcinoma in direct relation to smooth muscle, with no anatomic barrier to explain the circumscribed growth and lack of infiltration.

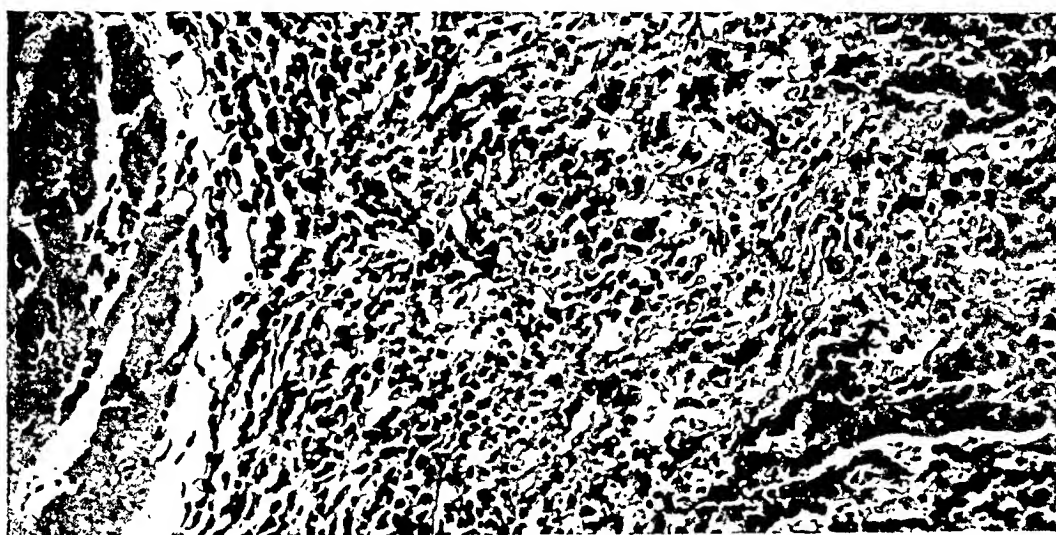
FIG. 8. Special undifferentiated carcinoma separated from muscle by an intense inflammatory reaction. Many of the advancing carcinoma cells show atrophy and pyknosis. Figure 14 illustrates the center of this tumor.

FIG. 9. Fibrous tissue and inflammatory reaction are interposed between this adenocarcinoma and the adjacent muscle. Some of the outer tumor cells exhibit atrophy and pyknosis. Another view of this same section (Fig. 11) reveals no similar anatomic barrier as an explanation for the circumscription.

7



8



9

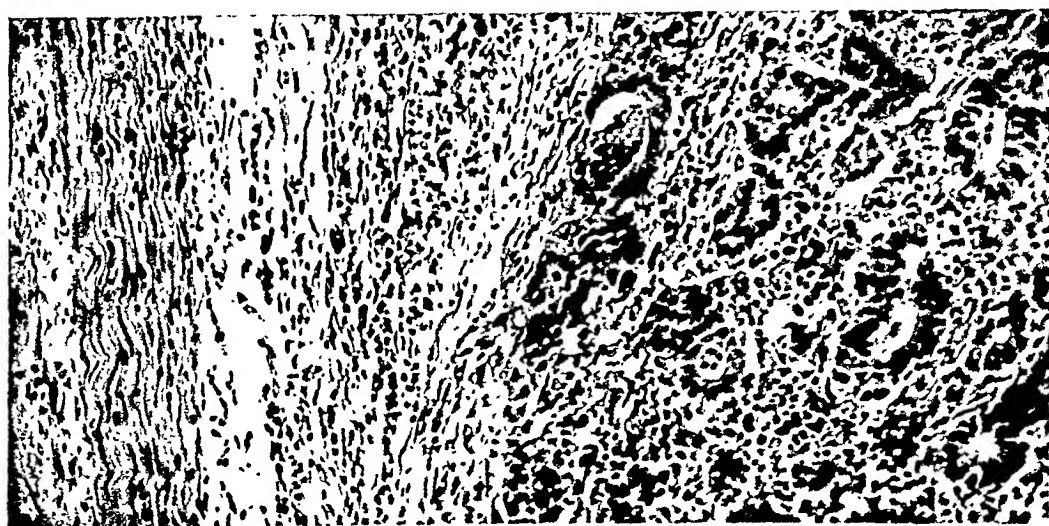


PLATE 157

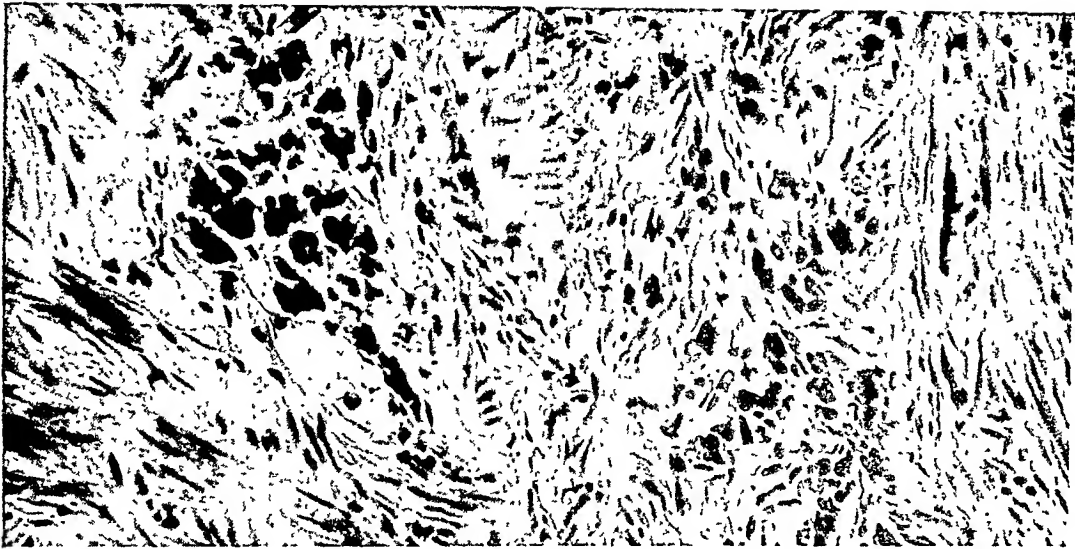
Three examples of degenerative changes in carcinoma cells as a possible explanation for the slow rate of growth. $\times 200$.

FIG. 10. Widespread infiltrating scirrhous carcinoma showing atrophy and pyknosis resembling that seen in prostatic cancers after orchiectomy.

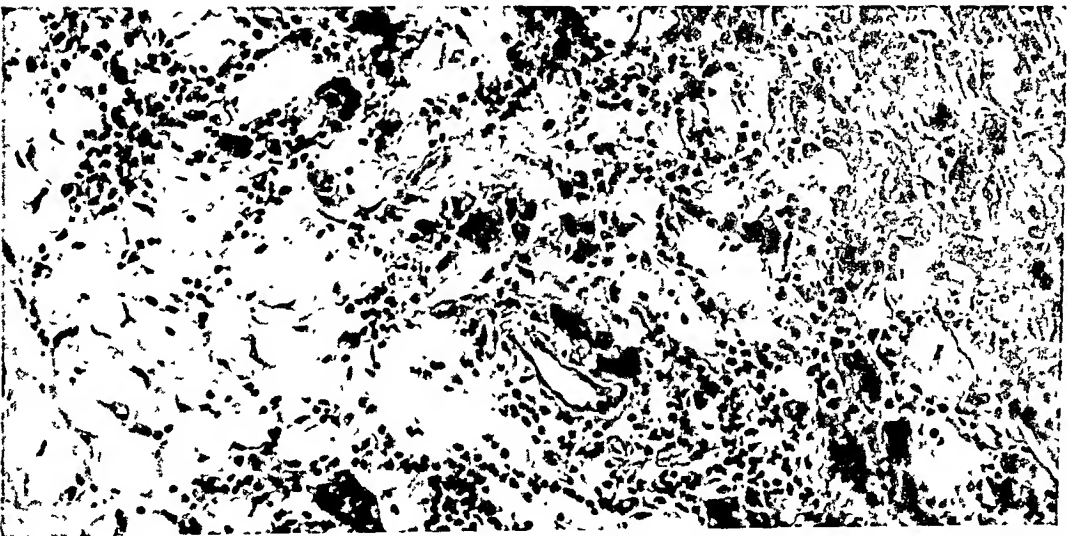
FIG. 11. Atrophic adenocarcinoma cells mixed with a few lymphocytes are found in edematous connective tissue beneath the muscularis mucosae. There is no anatomic barrier to infiltration by the cancer cells. Same case as shown in Figure 9.

FIG. 12. The adenocarcinoma cells at the margin of the tumor are smaller and darker than those farther back in the same gland.

10



11



12

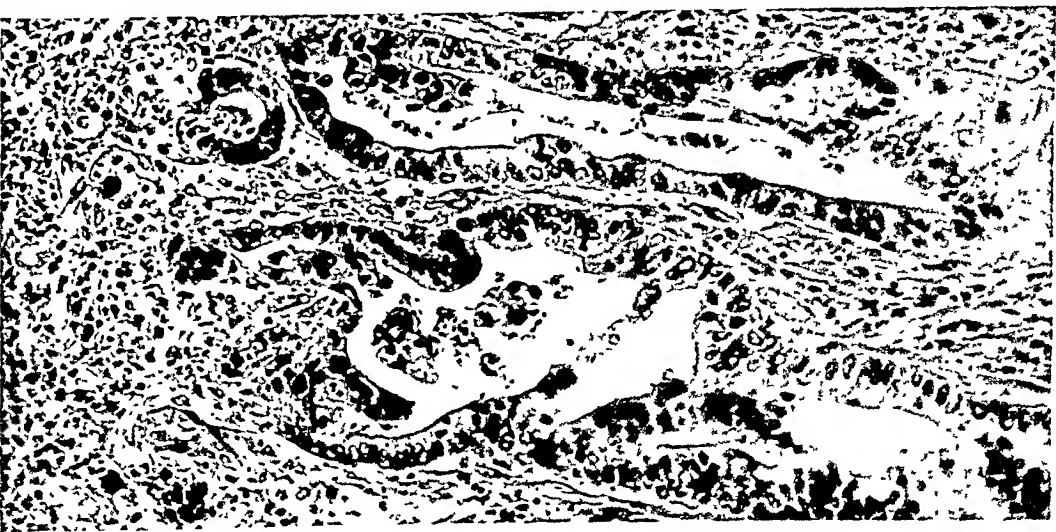


PLATE 158

Four of the more common histologic types of gastric cancer are represented in the 5-year survivors ($\times 200$). Two of these types (Figs. 14 and 15) are not present in the series of short-term survivors.

FIG. 13. Metastasis of an adenocarcinoma at necropsy 12 years after gastric resection, and 22 years after excision of a gastric adenoma malignum.

FIG. 14. Special undifferentiated carcinoma of nonscirrhous type. (Another view of this tumor is shown in Fig. 8.)

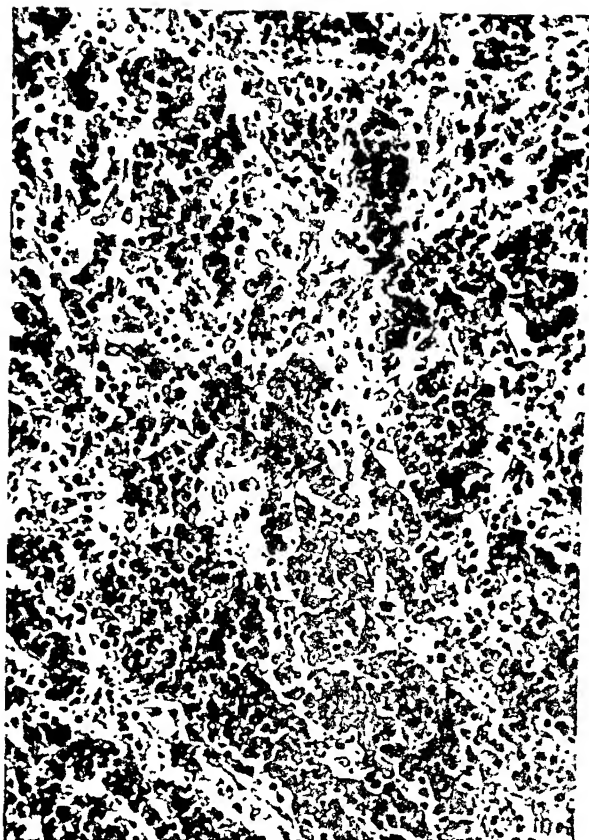
FIG. 15. Small-celled undifferentiated sarcoma or carcinoma, typical of 3 cases.

FIG. 16. Mucinous carcinoma, typical of 3 cases. (Low-power view is shown in Fig. 3.)

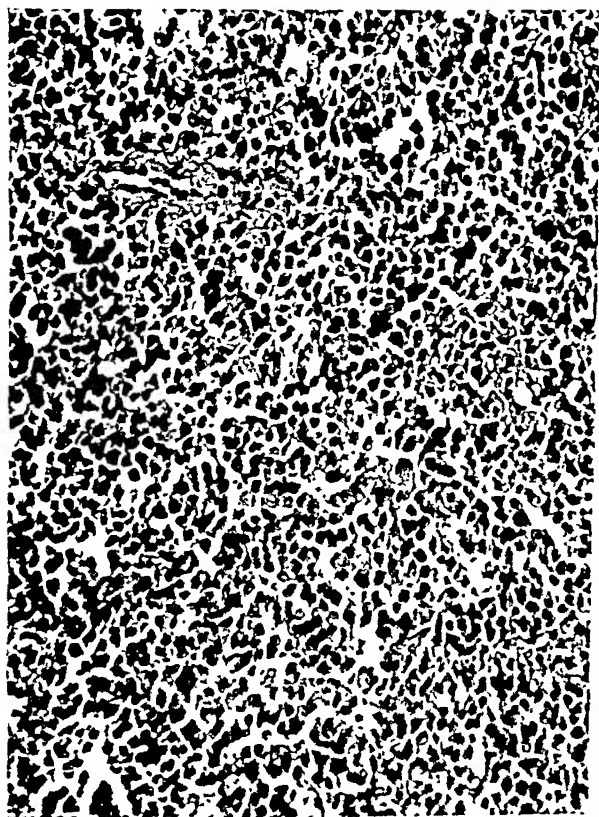
13



14



15



16



HISTOLOGIC FEATURES OF CARCINOMA OF THE CARDIO-ESOPHAGEAL JUNCTION AND CARDIA *

ELSIE McPEAK, M.D., and SHIELDS WARREN, M.D.

(From the Laboratory of Pathology, New England Deaconess Hospital, Boston 15, Mass.)

The greater part of our present knowledge of the growth and behavior of tumors is based on histologic studies. The importance of histologic classification of tumors in determining prognosis and therapy is obvious. The significance of the histogenesis of tumors is not generally so clear. It is possible that a more useful classification of tumors may result from studies directed toward the histogenesis of tumors of similar type in different locations. The advantage of a classification on this basis is exemplified by studies on adnexal carcinoma of the skin.¹⁻⁴

We are presenting a study of the histologic features of a series of carcinomas of the cardio-esophageal junction. This portion of the gastro-intestinal tract offers numerous possibilities for the origin of different types of carcinoma because of the presence of several varieties of epithelium: Stratified squamous epithelium of the esophageal mucosa, mucous epithelium of the free surface of the stomach and the gastric foveolae, the epithelium of the cardiac glands of the esophagus and the stomach, and the epithelium of the ducts and secretory portion of the esophageal glands. The statistics on incidence of carcinoma of the esophagus and of the stomach are not accurate, because of the listing of tumors of the cardio-esophageal portion with either organ. Notkin,⁵ in an attempt to determine the relative incidence of carcinoma of the cardia, found that such cases were included indiscriminately with carcinomas of both esophagus and stomach. He suggested isolating tumors of this region in a special group.

LITERATURE ON TYPES OF CARCINOMA OF THE ESOPHAGUS, STOMACH, AND CARDIO-ESOPHAGEAL JUNCTION

Carcinomas confined either to the lowest segment of the esophagus or to the proximal portion of the stomach generally carry the characteristics of the respective organ and are either epidermoid carcinoma or adenocarcinoma, but there are exceptions. Adenocarcinomas sometimes occur in the lower portion of the esophagus, seldom elsewhere.⁶⁻¹⁰ Adeno-acanthomas,^{11,12} basal cell carcinomas,^{8,13} and undifferentiated carcinomas^{7,14} of the esophagus also have been reported. Epidermoid carcinomas and adeno-acanthomas do occur in the stomach, but are

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extremely rare. In 1943 Wood¹⁵ reviewed the literature on tumors of the stomach which showed epidermoid characteristics and found 9 examples of pure epidermoid carcinoma and 9 of adeno-acanthoma of the stomach proper. To these he added 2 cases of adeno-acanthoma. In 276 consecutively resected carcinomas of the stomach, we have found 10 cases of adeno-acanthoma, 8 of which occurred at the cardio-esophageal junction and 2 in the pyloric region. We did not find a case of pure epidermoid type.

The carcinomas of the cardio-esophageal junction have not been presented as a special group in the past, but may be found reported with carcinomas of either the esophagus or the stomach. Wood¹⁵ did find reports of 5 cases of epidermoid carcinoma of this junction and one of adeno-acanthoma. An additional case of adeno-acanthoma¹⁶ and 3 cases of epidermoid carcinoma¹⁷⁻¹⁹ are found in the literature. Statements occur frequently in the literature as to the difficulty in determining the origin of tumors occurring at the junction between esophagus and stomach.¹⁹⁻²¹ Mallory¹⁹ stated that clinically or by roentgenologic examination it is impossible to distinguish the site of origin, that often it is impossible at autopsy, and that even histologic examination may not settle the issue. For this reason it would appear wise to isolate this group of tumors as Notkin⁵ suggested.

GENERAL REVIEW OF MATERIAL

A study has been made of 65 cases of carcinoma of the cardio-esophageal junction collected in the laboratory of the New England Deaconess Hospital from 1927 through part of 1947. These included 59 cases which definitely involved both stomach and esophagus, in addition to 6 cases which occurred so near the junction that involvement could not be ruled out except by serial sections. Fifty-seven of these tumors were resected specimens and 8 were obtained at autopsy. Most of the specimens were fixed *in toto* in Zenker's fixative. Multiple blocks were taken from various areas. All were stained with hematoxylin and eosin and many of them with eosin and methylene blue, van Gieson's stain, Papanicolaou's stain, phosphotungstic acid hematoxylin, Mallory's aniline blue, and Regaud's iron-hematoxylin stain.

A review of the gross aspects of these tumors provided no information for classification in addition to that already recorded for tumors of the esophagus and stomach. It is possible that in the future more detailed descriptions of the gross appearance of tumors in this region may reveal some features which can be correlated with the types isolated on histologic grounds. The chief feature of interest from review

of the gross appearance was the extent of involvement of esophagus or stomach as correlated with the histologic type.

Classification of the tumors on histologic grounds results in four groups: I, Epidermoid carcinoma; II, adeno-acanthoma; III, papillary adenocarcinoma with subgroups A and B; IV, miscellaneous, which includes adenocarcinomas and carcinoma simplex characteristic of carcinomas commonly found in other areas of the stomach. Table I shows the percentage of each group in the total series of carcinomas of the cardio-esophageal junction. Table II shows the extent of involvement of esophagus and stomach as found in the different

TABLE I
Types of Carcinoma at Cardio-Esophageal Junction and Cardia

Type	No. of cases	Percentage
Epidermoid carcinoma	2	3
Adeno-acanthoma (salivary gland type)	8	12
Papillary adenocarcinoma		
Subgroup A	3	5
Subgroup B	16	25
Miscellaneous	36	55
Total	65	

groups. It is evident that each of the groups can involve both esophagus and stomach. It is also to be noted that, relatively speaking, the first three types show a greater tendency to involve more of the esophagus and the fourth more of the stomach.

HISTOLOGY OF THE DIFFERENT GROUPS

Group I. Epidermoid Carcinoma

The epidermoid carcinomas were represented by only 2 cases. There were no unusual features in either, both following the usual pattern of pure epidermoid carcinoma of similar grades (epidermoid I and II) in any other region of the body. Both were resected specimens and both revealed metastases to regional lymph nodes. The metastases presented the same histologic pattern as the primary tumor.

Group II. Adeno-Acanthoma

In group II there were 8 cases. These tumors were tentatively classified as adeno-acanthomas, since that name describes the two most striking histologic patterns in these lesions. However, these particular tumors appeared to be different from adeno-acanthomas in other locations, as will be brought out in later discussion. It is seen in Table II that these tumors may involve esophagus and stomach to approximately the same extent, or the major portion of a tumor may

lie in either organ. The microscopic features were varied. The numerous types of histologic pattern may be summarized as follows.

1. Tumors with various epidermoid characteristics, as definite keratinizing cell masses with typical pearl formation (Fig. 1), single cell keratinization (Fig. 2), cells without keratinization but showing definite intercellular bridges, cells with markedly acidophilic cytoplasm (eosin), cells with large areas of vacuolization in the cytoplasm often containing a flattened pyknotic nucleus (Fig. 3), mosaic pattern of cell arrangement in which cell borders are very sharp and nests of cells with flattened nuclei are arranged in whorl-like formation suggesting early pearl formation (Fig. 2). Special stains aid in emphasizing the different characteristics common to keratinized epithelium,

TABLE II
Extent of Involvement of Esophagus and Stomach by Tumor

Type	Major portion in esophagus	Major portion in stomach	Equal involvement
Epidermoid carcinoma	0	1	1
Adeno-acanthoma (salivary gland type)	2	5	1
Papillary adenocarcinoma			
Subgroup A	1	2	0
Subgroup B	5	11	0
Miscellaneous	0	32	3

e.g., single cells with keratinization stain intense yellow with van Gieson's stain and orange with Papanicolaou's stain; phosphotungstic acid hematoxylin emphasizes intercellular bridges, mosaic pattern, and nests of cells in whorl formation.

2. Papillary adenocarcinoma, in which there are commonly large duct-like structures, usually with a very distinct basement membrane, containing varying amounts of proliferating papillary projections (Fig. 4). In some instances this proliferation is so exuberant and anastomoses and coalescence of papillary projections are so frequent as to form almost solid masses. The epithelium varies from low columnar to very tall cylindrical cells. The former usually have a large round or broad oval nucleus, and the latter a long, narrow, oval nucleus. Either type may show variation in staining, from an intense acidophilic color to an almost clear cytoplasm.

3. Mucinous carcinoma, in which the amount of mucin varies from extensive with large amounts in the stroma and no tumor cells visible to moderate with the glands partially lined by epithelial cells which are markedly distended with mucus.

4. Undifferentiated carcinoma, represented by cell masses of varying

size, in some instances composed entirely of round and polyhedral cells with rather indistinct cell borders and showing a light vesicular cytoplasm and relatively large nucleus. In other instances, similar cells are surrounded by a layer of tall cylindrical cells. Some of these masses appear to have been formed by the coalescence of papillary projections similar to those found in the glands of the papillary adenocarcinoma. There is often honeycombing of such masses by numerous small lumina which at times appear to be spaces left during coalescence of papillae and in other instances to be due to degeneration of a cell or cells to form small cysts.

5. Intimate admixture of different types of cells, with characteristics of keratinizing epithelium, glandular cells and undifferentiated cells (Fig. 5). There was variation in the extent of any one histologic pattern and in the degree of cellular differentiation from case to case. Extremes were represented by one example in which about 50 per cent of the tumor consisted of epidermoid carcinoma, grade I, about 35 per cent adenocarcinoma and undifferentiated carcinoma, and 15 per cent admixture of the three patterns; and by another case showing most of the tumor to be adenocarcinoma and undifferentiated tumor cell masses with honeycombing by numerous small lumina, with only very limited squamous cell differentiation represented by single cells showing early keratinization and vacuolization and by nests of cells in whorl-like arrangement. Four of the 8 cases in this group were autopsy specimens and 4 resected specimens. The 4 autopsy cases showed metastases to the following areas: Regional lymph nodes, pancreas, adrenals, liver, peritoneum, diaphragm, and lung. Two resected cases showed metastases in regional lymph nodes; one case showed no involvement of nodes, and the fourth was not accompanied by nodes. Metastases in most instances showed less differentiation of cells, both as to epidermoid characteristics and glandular differentiation, than was shown in the primary tumors.

Group III. Papillary Adenocarcinomas

There were 19 cases in the group of papillary adenocarcinomas and these were divided into two subgroups. *Subgroup A* consisted of 3 cases which showed portions of the tumor with a pattern of papillary adenocarcinoma, but in which the remainder of the tumor was different from the other 16 cases in this group, different from the usual stomach carcinoma and even different from other examples in this subgroup. *Subgroup B* consisted of 16 cases in which the outstanding histologic feature was that of a papillary adenocarcinoma which grew

into the wall with an inversion of the papillary structure. This inversion of the growth was in contrast to Borrmann's²² type I, papillary adenocarcinoma of the stomach.

Subgroup A

The three cases, placed together in subgroup A, showed the following histologic pictures in addition to the pattern of papillary adenocarcinoma in each.

Case 1 was an autopsy specimen in which the major portion of the tumor was in the esophagus. The papillary adenocarcinomatous pattern occurred only in the primary tumor. The remainder of the primary tumor and the metastases revealed glands lined by cuboidal cells, anastomosing cords of cuboidal cells, as well as small, solid masses of these cells in which tiny lumina appeared (Fig. 6). The glands were often lined by two layers of these cells. The cells in these areas were fairly uniform in size and shape, containing uniformly small, round, somewhat pyknotic nuclei. The stroma, particularly in the metastases, showed a somewhat myxomatous appearance. Mitotic figures were uncommon. The metastases were found in the liver, skin, subcutaneous tissue, and regional lymph nodes.

Case 2 was a resected specimen in which there was a very small region of papillary adenocarcinoma in the primary lesion. The remainder of the primary tumor and the metastases in the regional nodes showed anastomosing cords of cells, small nests of cells, and cells in adenoid arrangement (Fig. 7). These tumor cells were uniformly small and round with rather acidophilic cytoplasm (eosin) and contained uniformly small, round, centrally placed hyperchromatic nuclei. Mitotic figures were very rare.

Case 3 also was a resected specimen in which the papillary adenocarcinoma occurred in only a small portion of the primary tumor. The major part of the tumor was composed of solid, garland-like, broad strands of cylindric cells. The pattern seen in a part of the area of papillary adenocarcinoma which showed glands lined by tall, cylindric cells suggested that these solid strands of cylindric cells might be formed in some instances by coalescence of papillary projections. There was no tumor in the single node which accompanied the specimen, but carcinoma cells were present in the omental fat.

Subgroup B

Subgroup B was made up of 16 cases which were characterized by an inversion of the papillary structure, expansile type of growth, tendency to cyst formation, and presence of pseudo-stratification and

stratification of epithelial cells. The group was separated from the miscellaneous group on the basis of the following features:

1. Five of the 16 tumors involved largely the esophagus in the gross, as contrasted to the miscellaneous group, in which in not a single instance was the esophagus more involved than the stomach.

2. There was a marked tendency toward large duct-like structures (Fig. 10), often dilated to cystic proportions (Fig. 8), which showed a very distinct connective tissue wall in many instances that separated the tumor into adenoma-like areas (Fig. 9). The growth tended to be expansive rather than infiltrative, as is more common in the usual adenocarcinoma of gastric origin.

3. The tumor tissue of this group tended to involve the submucosal and muscular layers of the stomach rather than the mucosa.

4. The duct-like structures were lined by epithelium that was thrown into varying sized, finger-like branching projections supported by varying amounts of vascular strands of connective tissue. The epithelium sometimes outgrew the connective tissue, forming thick masses or convoluted layers which fused with one another. In such a manner, secondary lumina might honeycomb the more diffuse epithelial masses (Fig. 10). The resemblance of this type of growth to the areas of papillary adenocarcinoma of the adeno-acanthomas in the present series of tumors may be noted. Many times such portions gave the impression of papillomas that were inverted rather than everted and were sometimes referred to as pseudo-papillary tumors (Fig. 9).

5. The lining epithelial cells might be a single cell layer or they might be pseudo-stratified or even stratified in small areas. In either case they might be tall and cylindric or cuboidal, but they tended to be of uniform size (Figs. 11 and 12).

6. The cell outlines were moderately sharp, and there was retention of polarity and basement membrane. Even in the portions with exuberant growth, the cells tended to be more sharply defined than in the more characteristic syncytium-like growth of the usual gastric carcinoma (Figs. 10, 11, and 12).

7. Occasionally a few cells were in whorl-like arrangement, a pattern seen in the adeno-acanthomas.

8. Variable staining reaction of the cytoplasm of the epithelial cells was present, but there was a tendency toward strongly acidophilic staining (eosin). Rarely, this might be so marked in a single cell as to suggest early keratinization.

9. The nuclei were, on the whole, uniform in shape and size, and often lay toward the base of the cells. Most nuclei were long and oval, and many were extremely thin and hyperchromatic, almost pyknotic.

They were relatively small in comparison to the amount of cytoplasm (Figs. 11 and 12). Mitotic figures usually were infrequent, though in some tumors they were more numerous.

10. The cysts usually contained masses of granular, hyalin-like material as well as large numbers of squame-like forms (Fig. 11). The latter apparently represented the desquamation of the long, thin nuclei noted above (stained blue with iron hematoxylin).

11. Necrosis of small cell groups with small cyst formation was common. More extensive necrosis of larger areas of the tumor was not infrequent.

12. Extensive inflammation characterized by large masses of polynuclear cells, lymphocytes, and plasma cells was not infrequent.

13. Mucinous secretion of extensive degree occurred in some tumors. Often there were large cysts lined by tall, cylindric cells distended with mucus. The lumina of the cysts might contain a few cells as well as masses and strands of mucus which often diffused into the surrounding stroma. This type of mucinous secretion was seen in gastric carcinoma, but was somewhat less frequent than is the signet-ring type of carcinoma.

The tumors in this group showed the above characteristics in both the primary growth and the metastases. In the 4 cases on which an autopsy was performed, only one showed distant metastases. In all of the 16 tumors in this group, the glandular differentiation was between 75 and 100 per cent, with 100 per cent in most instances. In some there were small glands like those seen in the usual gastric carcinoma. A sharp line between this group and highly differentiated adenocarcinomas of definite gastric origin could not be drawn, but the above characteristics appeared to be distinct enough to separate cases of that type for further study as to their behavior.

Group IV. Miscellaneous Group

The miscellaneous group of 32 cases included all of the adenocarcinomas that did not fall into group III, subgroup B, as well as the cases of carcinoma simplex and of mucinous carcinoma. It is not necessary to describe in detail all of the variations seen in this group, but merely to point out differences between the glandular tumors of this group and group III, subgroup B. Most of the carcinomas of well differentiated glandular type were of three general types: (1) Papillary form (9 cases), also known as Borrmann's²² type I or fungating carcinoma, which grew into the lumen of the stomach rather than into the wall, in contrast to those of group III, subgroup B, which we have called papillary adenocarcinoma. (2) Adenocarcinoma of a high degree of glandular differentiation resembling intestinal adenocarcinoma

(5 cases), which were similar to group III, subgroup B, in their sharp delineation of glands with uniform epithelial lining cells and retention of cell polarity and of basement membrane. They nearly always showed a single layer of epithelial cells lining the glands. Duct-like structures and cyst formation were not seen. (3) Adenocarcinomas with varying amounts of glandular differentiation, in which glands were variable in size and, as a rule, were markedly tortuous. Sometimes there was cyst formation in portions of these tumors and then it was often difficult to separate such a tumor from the papillary adenocarcinomas of group III, subgroup B.

In the miscellaneous tumors, there were 18 cases which showed 75 to 100 per cent glandular differentiation. The other cases in this group showed varying amounts of differentiation to glandular form and included a few diffuse infiltrating carcinomas lacking any gland formation.

EMBRYOLOGY AND HISTOLOGY OF CARDIO-ESOPHAGEAL JUNCTION

The esophagus, stomach, and respiratory systems all develop from the entodermal layer in the form of a hollow tube. The differentiation of these structures from each other takes place at different stages of development, being completed by the seventh week. At first this tube is lined by a simple layer of low-columnar epithelium. Following the separation of the esophagus, stomach, and respiratory system, the epithelium of the esophagus as well as of the respiratory system is transformed into ciliated epithelium. Then in the esophagus clear, vesicle-like, glycogen-containing elements appear between the ciliated cells. Later these glycogen-containing cells are transformed into superficial squamous cells and partly into ciliated cells. Finally the ciliated cells are reduced in number and are usually completely desquamated at birth, leaving the esophagus lined by stratified squamous epithelium.^{23, 24}

The esophagus contains two types of glands, esophageal glands which are found in the submucous layer and superficial glands, termed "cardiac glands," found in the lamina propria mucosae. The esophageal glands are entirely mucus-producing glands.²⁵ They are formed by epithelial cells growing out from the surface epithelium through the muscularis mucosae in the form of ducts with subsequent glandular development in the submucosa.²⁶ These structures occur at all levels of the esophagus, vary greatly in number and distribution in different individuals, and often give rise to cysts.²⁵ The glands are compound with branched tubulo-alveolar terminal portions containing purely mucous epithelium. The epithelium lining the smallest ducts is cuboidal or low-columnar, becoming stratified in the larger ducts, and in the en-

larged main duct the lining is made up of stratified squamous epithelium.²⁴ These glands are identical with the small mucous glands of the oral and nasal cavities, pharynx, larynx, trachea, and bronchi, some of which are considered to be salivary glands of the mucous type.²⁷

The "cardiac glands," given that name because of their resemblance to the cardiac glands of the stomach, are found also at different levels in the esophagus,²³ although they are more prominent at the level of the cricoid cartilage and at the point of transition of esophagus and stomach.²⁸ The terminal portions have branched and curled tubules containing columnar or cuboidal cells which give the mucin reaction. The excretory ducts are lined by tall-columnar cells similar to the mucous epithelial cells of the gastric foveolae.²⁴ It is probable that in the descent of the stomach a few rests of cells that are destined to become gastric mucosa remain in the esophageal portion of the gut and become islets of gastric mucosa.²³ At times these glands contain a few parietal cells or zymogenic chief cells which, according to Bensley,²⁹ indicates, along with other features, that these glands represent decadent or retrogressive structures derived from fundus glands.

The "cardiac glands," as described in the esophagus, extend varying distances into the stomach. In our present study we will confine our interest to this zone. These glands blend gradually with the fundic glands, which contain numerous parietal and zymogenic chief cells. The other types of epithelium of interest in the stomach immediately adjacent to the esophagus are the surface epithelium of tall-columnar, mucus-producing type like that in the ducts of the "cardiac glands" and occasional patches of stratified squamous epithelium which replace the surface epithelium. These patches of squamous epithelium may represent suppression of the gastric glands with replacement of the mucigenous epithelium, as is thought to occur in rodents and ruminants.²⁹ According to Kirk³⁰ and Lim,³¹ the mucous cells of the surface of the stomach are probably the most primitive of all gastric epithelia, with mucous cells of the cardiac and pyloric glands a little more specialized and with the highest cyto-differentiation shown in the parietal and zymogenic chief cells.

HISTOGENESIS OF CERTAIN TUMORS OF THE CARDIO-ESOPHAGEAL JUNCTION

Group I. Epidermoid Carcinomas

The origin of the pure epidermoid carcinomas of the cardio-esophageal junction has been attributed to the squamous cell epithelium of the esophagus.¹⁷ Such tumors could arise in the squamous

epithelial patches in the stomach and extend up to involve the junction. Or they could originate in the glandular portion of stomach mucosa, since such tumors do occur in the stomach at sites too distant from the junction to be attributed to squamous epithelial patches.¹⁵ The histogenesis of the latter type of epidermoid carcinoma is discussed by Wood.¹⁵ Since both of the epidermoid tumors in this series are straightforward keratinizing tumors, the more likely origin is in the esophagus.

Group II. Adeno-Acanthomas

The 8 cases of carcinoma of the cardio-esophageal junction which we have called adeno-acanthomas were given that name on the basis of the combination of glandular cells and cells with characteristics of keratinizing epithelium. We are convinced that the comparison of these tumors with our 2 cases of adeno-acanthoma of the pyloric region of the stomach shows a difference in the histologic pattern and possibly in the mode of origin. We believe the adeno-acanthomas in our group represent tumors to be classified with salivary gland tumors of other regions of the body. These tumors are similar to an adeno-acanthoma of the esophagus which we have reported recently.¹¹ This case showed certain features which differed from adeno-acanthomas in the stomach (pyloric region),¹⁵ intestine,³² and gallbladder.^{33,34} We expressed the opinion in that report that this tumor probably arose in the esophageal glands, which are mucous glands of the salivary gland type. Reports such as Ahlbom's³⁵ in 1935 on tumors of the mucous-serous glands of the mouth and Ringertz'³⁶ in 1938 on similar tumors of these glands in the nasal cavities, accessory sinuses, and nasopharynx emphasized the importance of these glands as a source of carcinoma.

The reader is referred to these authors for comparison of the histologic features and mode of development of these tumors, but, in brief, Ahlbom³⁵ stated that such tumors show widely varied patterns, sometimes in a single case, e.g., tumors that are mainly adenocarcinoma often show cylindromatous features, papillary cystic structures, or solid epithelial areas resembling basal cell carcinoma or even squamous cell carcinoma; and that squamous epithelial types usually show one or more additional structural types. Often metastases, according to Ahlbom, may differ from most of the primary tumor, though usually the structure occurs in the primary tumor to some extent. Ringertz³⁶ also described widely varying patterns that are found in salivary gland tumors, even several histologic patterns in one case. It is this tendency to multiplicity of patterns in a single case that is convincing evidence for classification of these 8 cases of combined glandular and squamous cell carcinomas as salivary gland tumors. In the adeno-acanthomas

found in other locations, the pattern is similar to the picture produced by areas of squamous cell metaplasia in normal glandular epithelium. The single cell keratinization, the whorl-like nests of cells, mosaic pattern, and vacuolization of cells are lacking. Of course, that type of adeno-acanthoma could occur also at the cardio-esophageal junction, but we have no cases of this type.

In these 8 cases of carcinoma of salivary gland type of the cardio-esophageal junction and in the case of esophageal adeno-acanthoma mentioned above we have been unable to trace direct origin to any portion of the esophageal glands. Little emphasis has been placed on the submucous glands of the esophagus as a source of carcinoma. Clayton¹³ reported an adenocarcinoma of the upper one-third of the esophagus, which he attributed to the esophageal glands. Ahlbom³⁵ stated that rare reports of basal cell carcinoma of the esophagus may represent tumors of these glands. He referred to a case report of a mixed tumor of the esophagus³⁷ which he believed might be of the salivary gland type with its origin in the esophageal glands. A rare case report of an adenocarcinoma of the esophagus in regions where the cardiac glands are infrequent suggests that the esophageal glands can give rise to carcinoma.^{13, 38} Recently in our laboratory we reviewed a case of resected carcinoma of the esophagus in which the upper portion of the tumor began at the level of the aortic arch and involved 3.5 cm. of the esophageal wall below this level. This tumor showed areas of typical epidermoid carcinoma, grade I, in addition to several carcinomatous glands. In one area there were ducts typical of esophageal gland ducts, which showed early carcinomatous changes. This case is strong support for directing attention to the esophageal glands as a possible source of carcinoma.

Group III. Papillary Adenocarcinoma

Subgroup A

Again in the papillary adenocarcinomas we have tumors which show a combination of patterns. Each tumor revealed a limited area of papillary adenocarcinoma similar to that seen in many of the adeno-acanthomas of group II. In addition, there is an entirely different histologic picture from that of any other tumor of the entire series. The first case, as can be noted from previous description, showed a pattern in the primary growth as well as in the metastases that was very similar to that seen in salivary gland tumors both of the mixed type and of the type seen more frequently in the mouth and nasopharynx, which has been termed basalioma.^{35, 36} The second case had a somewhat similar pattern although not as characteristic; but the

marked uniformity of cells as to size, shape, and arrangement, as well as similar uniformity of nuclei, set this apart. Perhaps it represents an undifferentiated carcinoma of the salivary gland type.³⁶ The third case showed a structural form in the major portion of the tumor that could be compared with Ringertz'³⁶ solid cylindric cell carcinoma which he found fairly frequently in the nasopharyngeal region. These tumors, he believed, may start as inverted papillary projections of cylindric epithelium in which lumina are formed and then become filled with proliferating tumor cells so that broad, solid strands of cylindric epithelium are formed. Branching of such strands and further coalescence may occur. The epithelium tends to grow in stratified layers and early squamous cell differentiation is not uncommon. However, he stated that the origin of such tumors is not entirely clear-cut, since they might start from surface epithelium of the nasopharynx (columnar epithelium) as well as from ducts of salivary glands, whereas the basalioma type appears to have clear-cut origin in the glandular part of the salivary glands. In line with the possibility of origin from surface epithelium, Kaufmann³⁹ referred to a solid cylindric cell carcinoma of the stomach described by Hauser, which Kaufmann stated might be classed as the basal cell carcinoma of cylindric epithelial mucosae described by Krompecher. Kaufmann considered such tumors rare, and certainly recent literature does not mention them. It is of interest that the first case in this group showed the major portion of the tumor in the esophagus. It would appear that in the first 2 cases the most likely source was the esophageal glands and that in the third case it could have been in these glands or possibly in the cylindric epithelium of the "cardiac glands" or surface mucosa of the stomach.

Subgroup B

The tumors of subgroup B which have been described as to certain specific characteristics could arise in several different sites: Mucous epithelium of the free surface of the stomach, the gastric pits or necks of gastric glands, the tall-columnar epithelium of the excretory ducts of the "cardiac glands" of the esophagus, ducts of the esophageal glands, or possibly from the terminal portions of any one of the glands. It is not unlikely that different tumors originate in all of these sites, although the pattern does not suggest the last source listed. However, Ringertz³⁶ pointed out that carcinoma of cylindric cell type and adenocarcinoma supposed to arise in the glandular epithelium cannot be sharply separated. MacCallum⁴⁰ mentioned the tendency for tumors of the cervix uteri to develop near the line of transition of stratified into cylindric epithelium. Ewing⁴¹ also referred to such transition

zones in the esophagus as being a predisposing factor for epithelioma. At the cardio-esophageal junction there is ample opportunity for such factors since the transition zones are multiple: Surface squamous epithelium of esophagus to surface mucous epithelium of stomach, and several such areas may appear when there are patches of squamous epithelium in the stomach; squamous epithelium to columnar epithelium of the ducts of the cardiac glands; and squamous epithelium to stratified cuboidal and columnar epithelium in the ducts of the esophageal glands.

Proof of any specific origin for any one tumor of this group is not possible. This is particularly evident when we find similar tumors described as gastric tumors of other areas. Ewing⁴¹ mentioned a tumor of stomach origin, described by Hauser,⁴² which he called carcinoma cylindro-cellulare microcysticum which resembled closely some carcinomas of this group. However, this author stated that such tumors are not common and are usually found at the pylorus. On the other hand, Ahlbom³⁵ and Ringertz³⁶ in their classifications of types of salivary gland tumors included papillary cystic adenocarcinomas. It is not infrequent to find combinations of papillary cystic adenocarcinoma, alveolar carcinoma, and mucinous carcinoma in a single salivary gland tumor; but such combinations can be found also in carcinomas of gastric origin. Thus the only conclusion can be that we have an adenocarcinoma that tends to be an inverted, somewhat circumscribed papillary tumor, often with cystic degeneration, showing certain specific features in the epithelium, that may arise in the esophageal glands as well as in other tissues. The presence of areas of a very similar type of papillary adenocarcinoma in tumors of group II points to the esophageal glands as a source of the tumors just discussed.

Group IV. Miscellaneous Group

In the miscellaneous group are included all other glandular tumors, as well as the examples of carcinoma simplex and mucinous carcinoma. These tumors are of the same types as those commonly found in the stomach at other sites. The line between those which show a high percentage (75 to 100 per cent) of glandular differentiation and the tumors of group III, subgroup B, cannot be sharply drawn. There will be no attempt to trace specifically the histogenesis of these tumors, since neither the literature nor our study permits any very definite theories. Stout⁴³ stated that most carcinomas of the stomach are derived from mucous epithelium. Whipple and Raiford⁴⁴ indirectly suggested the origin of carcinoma of the stomach in other than the mucous cells. They found that the majority of gastric carcinomas

differ from the carcinomas of other parts of the gastro-intestinal tract, that they are less well differentiated and are therefore more malignant. Such a difference they believed may be due to a greater variety of type and function of epithelial cells of gastric mucosa. Other observers⁴⁵ have made similar statements.

It is, however, interesting to record a few points about some of these tumors which may suggest certain theoretic considerations as to specific origin. In this miscellaneous group, 18 cases, or 50 per cent, showed glandular differentiation of 75 to 100 per cent. If we add the 16 cases of group III, subgroup B, also differentiated to this degree, the percentage of those so differentiated is raised to 65.4 per cent of the 52 tumors. In a review of 20 cases of gastric carcinoma of other

TABLE III
Carcinomas with 75 to 100 Per Cent Glandular Differentiation

Type	No. of cases	No. of cases with 75 to 100% glandular differentiation	Percentage with 75 to 100% glandular differentiation
Papillary adenocarcinoma, subgroup B	16	16	100
Miscellaneous group	36	18	50
Total	52	34	65.4

areas, we found only 25 per cent with this degree of differentiation. Schindler, Steiner, Smith, and Dailey⁴⁵ in grading 50 cases found only 28 per cent and Dochat and Gray⁴⁶ found 33 per cent of 1,045 cases. Borrmann's²² type I carcinoma is represented in the present series by 9 cases, or 14 per cent (2 cases of squamous cell carcinoma excluded), or 16 per cent if we exclude groups I and II. This is contrasted with 20 cases, or 9.5 per cent, of this type I in 211 cases of carcinoma in other parts of the stomach. In addition, there were 5 cases in the group that resembled adenocarcinoma of the large intestine of a type that often reminds one of a simple adenoma.

Since a high level of glandular differentiation is considered to be an indication that tumors will reproduce more nearly normal tissue, we might expect tumors derived from epithelial cells of simple type and less complicated function to reproduce the normal pattern more frequently. We noted under the review of the embryology and histology of the cardio-esophageal area that the cells of the surface mucous epithelium of the stomach and of the mucous epithelium of the cardiac and pyloric regions are more primitive (and their function probably less specialized) than the cells of the gastric glands of the fundus and antrum. Thus the high percentage of more highly differentiated tumors

in the cardio-esophageal junction may point toward origin in the more simple mucous epithelium, whereas the more anaplastic carcinomas, found more frequently in other areas of the stomach, may represent tumors arising in cells with more highly specialized function.

BEHAVIOR OF TUMORS OF THE CARDIO-ESOPHAGEAL JUNCTION

Definite conclusions as to the behavior of these tumors cannot be drawn on such a small series. In the future, however, as more tumors are studied from this region, the statistical evidence may be increased to figures which are significant.

In Table IV, the average age and the sex is recorded for each group.

TABLE IV
Sex and Age Distribution of the Different Types of Carcinoma

Sex	Epidermoid carcinoma	Adeno-acanthoma (salivary gland type)	Papillary adenocarcinoma		Miscellaneous	Total no. cases	Percentage
			Subgroup A	Subgroup B			
Males	1	5	3	15	26	50	77
Females	1	3	0	1	10	15	23
Average age	56 yrs.	62.5	52	55	58.5	56.8	

As is evident, there was no striking difference between any of the groups as to age incidence. The average age in a series of 225 carcinomas of the stomach from other areas, collected in our laboratory, is 56.8 yrs. The age range for carcinoma of the esophagus and stomach is quoted as commonly between 50 and 60 years of age, although either can occur at a much younger or older age level.^{6, 47, 48, 49} Ahlbom³⁵ reported the average age for malignant salivary gland tumors as 52 years, but it may be that those of the type similar to the tumors in the present series would show a different age level.

Males outnumbered the females in each group, and in the entire series there are three times as many males as females. This again is in accord with the reported higher incidence of carcinoma of the esophagus and stomach in the male.^{6, 48, 49, 50} Ahlbom³⁵ found no significant difference in sex incidence of salivary gland tumors. In our group II, which is the group most like the salivary gland tumors, the ratio was 5 males to 3 females; but this group is too small for evaluation.

In Table V is found the percentage of incidence of metastases in the entire series of cardio-esophageal carcinoma, as well as in each group.

The percentage of metastases for carcinoma of the esophagus, which

is not broken down as to type, was quoted by Watson ⁶ as 71.5 per cent, and by Adams ¹⁰ as 65 per cent. For metastasis from gastric carcinoma, Stout ⁴³ found 89.5 per cent of 143 autopsied cases; Whipple and Raiford, ⁴⁴ 55.7 per cent of 95 resected cases; Dochat and Gray, ⁴⁶ 59 per cent of 1,045 cases, most of which were autopsied. Our figures on 223 cases of resected gastric carcinomas, excluding the present series, is 64.7 per cent. As a group the cardio-esophageal tumors metastasize to the same degree, 66 per cent, as the gastric carcinomas of other portions of the stomach. It is to be noted, however, that when the series

TABLE V
Frequency of Metastases as to Histologic Type of Carcinoma

Type	No. of cases	Resected specimens	Autopsies		No. with metastases	Percentage with metastases
			Operated	Not operated		
Epidermoid carcinoma	2	2	0	0	2	100
Adeno-acanthoma (salivary gland type)	8	4	0	4	6	75
Papillary adenocarcinoma						
Subgroup A	3	2	0	1	2	66.6
Subgroup B	16	15	3	1	9	56.2
Miscellaneous group	36	34	8	2	24	66.6
Total	65	57	11	8	43	66.1

is broken down into the groups there are some differences which may be significant, even though some groups are very small. Both cases of epidermoid carcinoma showed metastases, 6 of the 8 cases in group II, or 75 per cent, and 9 of the 16 cases in group III, subgroup B, or 56 per cent. As for metastases from salivary gland tumors, it is not easy to compare our findings with previous studies since the location again has something to do with the behavior of the tumor. Ahlbom ³⁵ stated that of the malignant salivary gland tumors, those occurring in regions other than the large salivary glands are more malignant. In general, the group as a whole shows a low percentage of metastases, 25 per cent. But it is to be noted that Ahlbom referred to those salivary gland tumors of undifferentiated type and those showing squamous cell differentiation as the more malignant and stated that these metastasize frequently. This difference in the malignancy of different types of salivary gland tumors was emphasized also by Quattlebaum, Dockerty, and Mayo.⁵¹ Another point of interest is Ahlbom's ³⁵ statement that the salivary gland tumors of papillary-cystic type are relatively benign, although some are more malignant than others and these are often more papillary than cystic.

There are only 11 cases of the resected carcinoma of the cardio-esophageal junction for which we have the final findings. In Table VI the occurrence of metastases is given for these cases at autopsy, as well as for 8 other autopsied cases included in the series.

The incidence of metastases in the 19 autopsied cases is 14, or 73.7 per cent. Of the 6 autopsied cases on which gastric resection had been done, only one showed distant metastases on necropsy. This finding is important in the consideration of treatment of carcinoma in this area. All deaths on the resected cases were due to postoperative complications, but all operations in these cases were performed before 1946.

COMMENT

Sixty-five cases of carcinoma of the cardio-esophageal junction have been described and analyzed with reference to their possible histogenesis. The information gained from the review and its correlation

TABLE VI
Occurrence of Metastases at Autopsy

Type of tumor	No. of cases	Operated		Without operation	
		Metastases	No metastases	Metastases	No metastases
Epidermoid carcinoma	0	0	0	0	0
Adeno-acanthoma (salivary gland type)	4	0	0	4	0
Papillary adenocarcinoma					
Subgroup A	1	0	0	1	0
Subgroup B	4	2	1	1	0
Miscellaneous group	10	4	4	2	0
Total	19	6	5	8	0

with similar studies in the literature point to the origin of the adeno-acanthomas and the 2 cases of papillary adenocarcinoma, subgroup A, in the esophageal glands of the esophagus. These glands are to be classed as similar to the salivary glands of the mouth and nasopharyngeal region. There is an additional group of tumors, one case of papillary adenocarcinoma, subgroup A, and all the cases of papillary adenocarcinoma, subgroup B, that may arise in the same type of gland, but the origin of some of these may be in the "cardiac glands" of the esophagus or in the stomach mucosa.

There appears to be some evidence that more well differentiated glandular tumors occur at the cardio-esophageal junction than in other parts of the stomach. It is suggested that this may be due to the presence of gastric epithelium in this area that is of a more simple type. The tumors of papillary adenocarcinoma, subgroup B, show a tendency

to an expansive rather than an infiltrative type of growth with frequent cystic changes which may have some significance in the lower percentage of metastases in these tumors than in any other group.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 159

FIG. 1. Area of keratinizing cell masses with pearl formation. Hematoxylin and eosin stain. $\times 250$.

FIG. 2. Cells in mosaic pattern with keratinization of a few cells. Hematoxylin and eosin stain. $\times 400$.

FIG. 3. Marked vacuolization of cells with flattened pyknotic nuclei. Hematoxylin and eosin stain. $\times 400$.

FIG. 4. Papillary adenocarcinoma. Hematoxylin and eosin stain. $\times 250$.

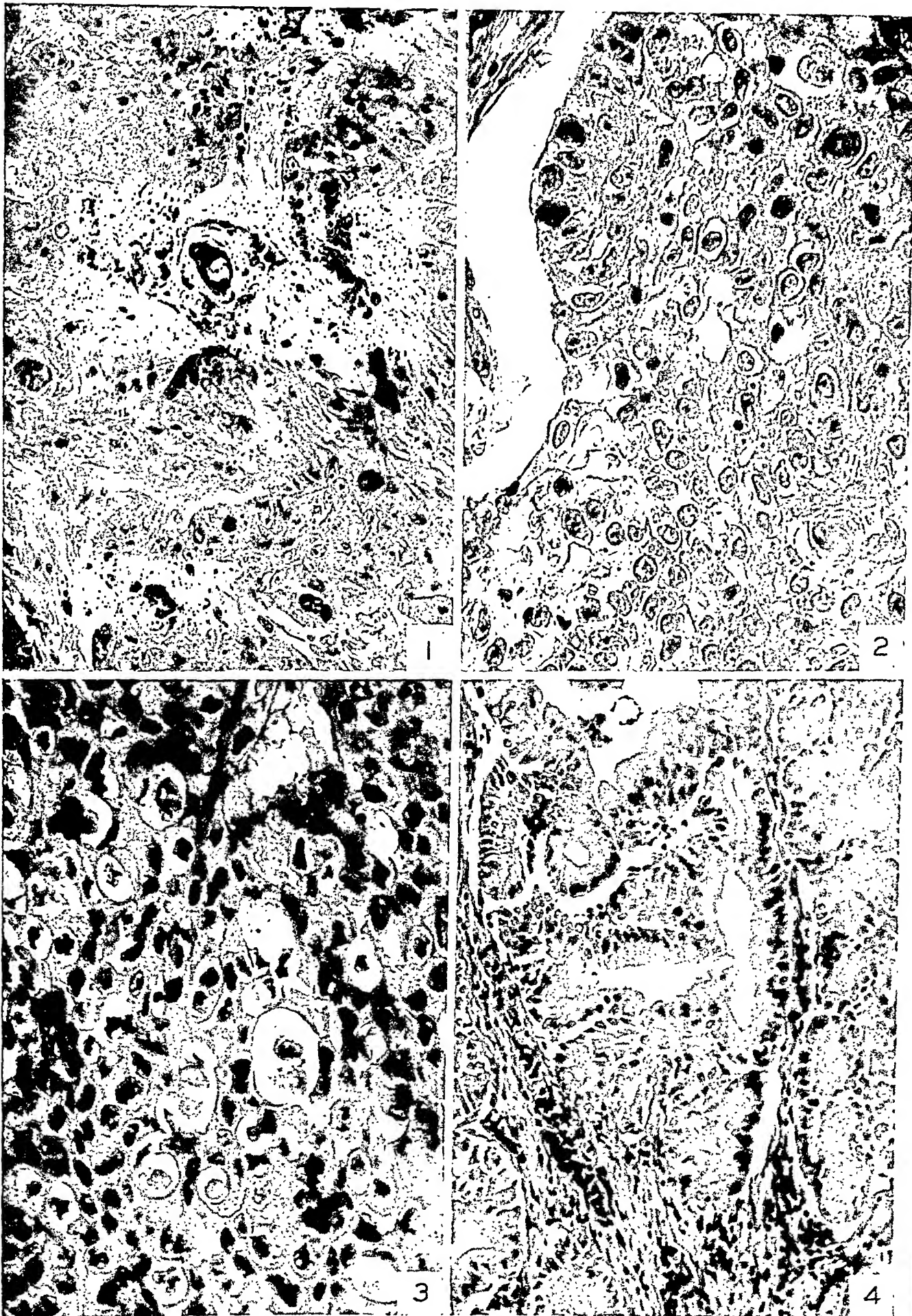
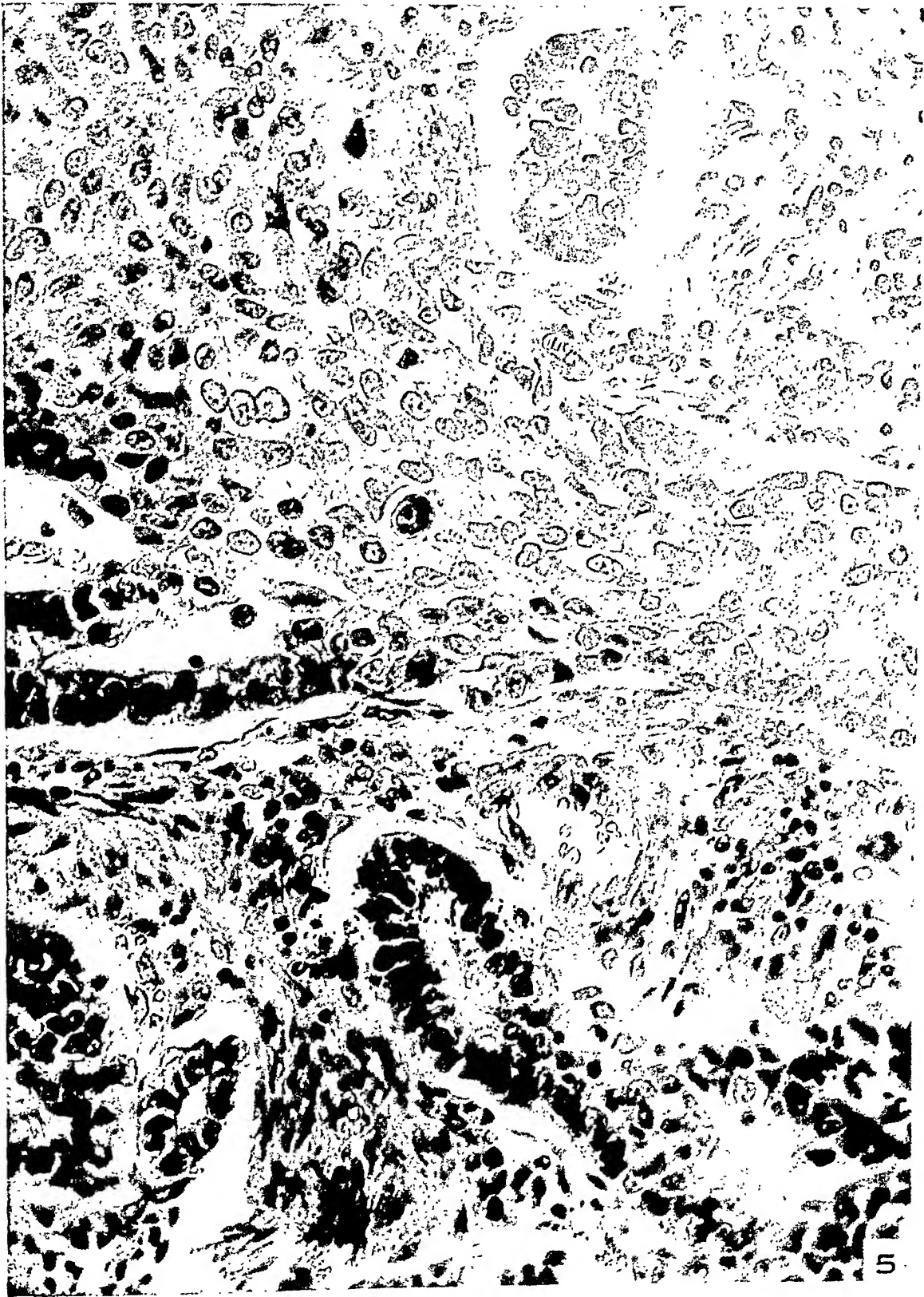


PLATE 160

FIG. 5. Admixture of undifferentiated cells and glandular cells with single cell keratinization. Papanicolaou stain. $\times 400$.



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Cardio-Esophageal Carcinoma

PLATE 161

FIG. 6. Anastomosing cords and masses of cuboidal cells containing lumina in a myxomatous stroma. Case 1, group III, A. Hematoxylin and eosin stain. $\times 125$.

FIG. 7. Anastomosing cords and nests of small cells. Case 2, group III, A. Hematoxylin and eosin stain. $\times 250$.

FIG. 8. Cysts characteristic of group III, B. Hematoxylin and eosin stain. $\times 125$.

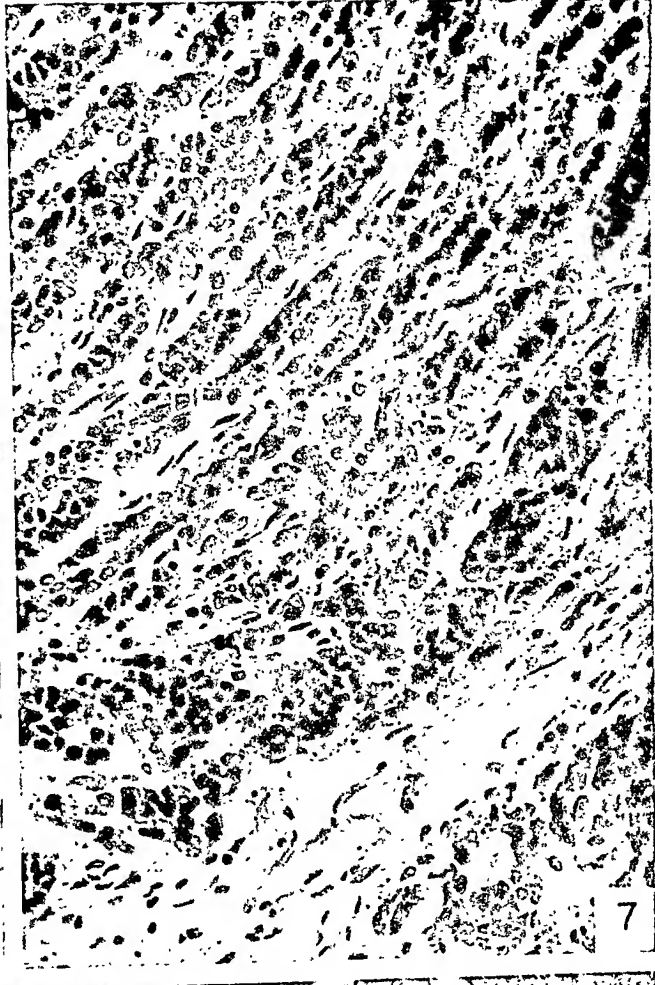


PLATE 162

FIG. 9. Expansile adenoma-like growth of carcinoma. Iron hematoxylin-van Gieson stain. $\times 35$.

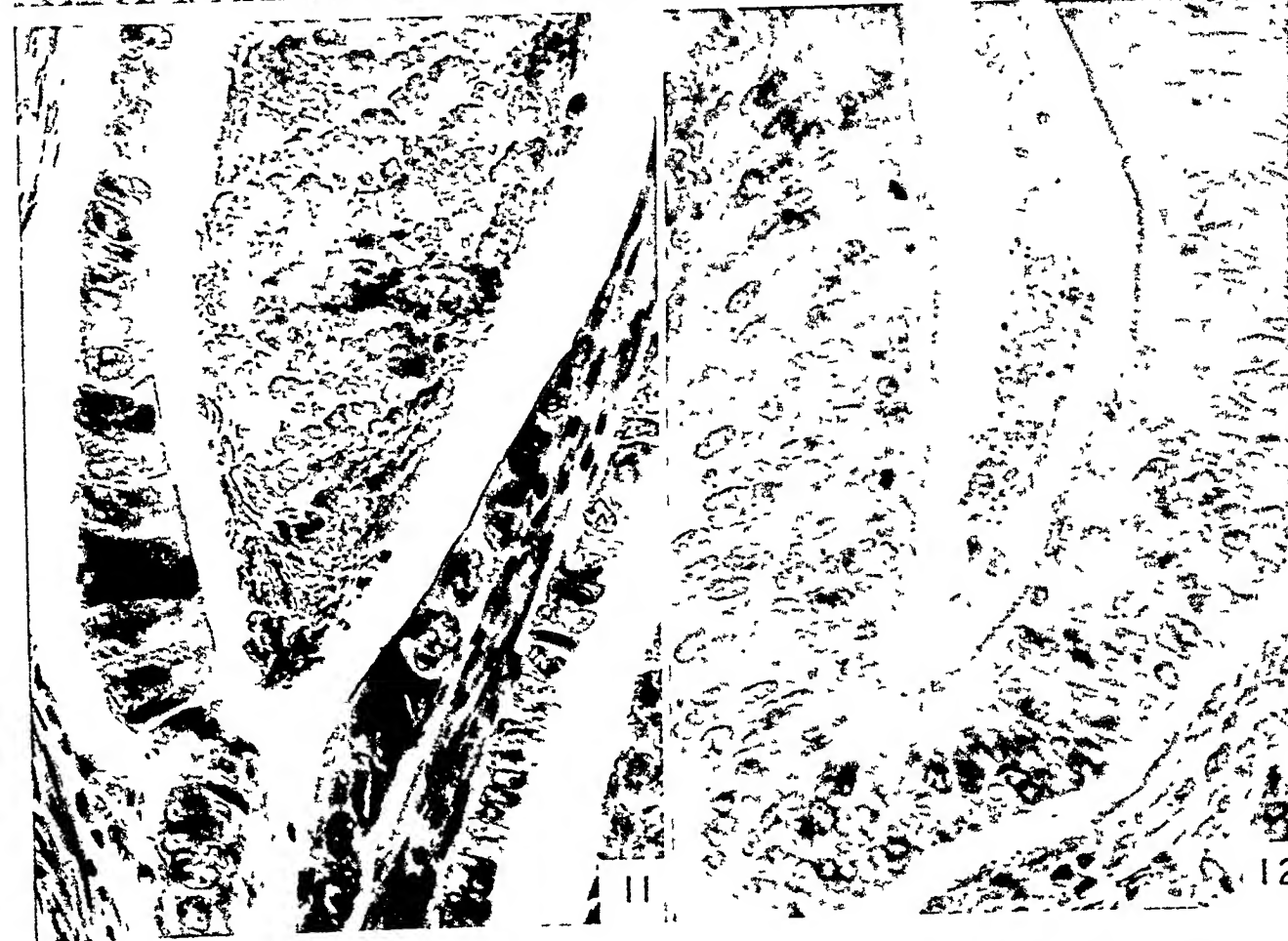
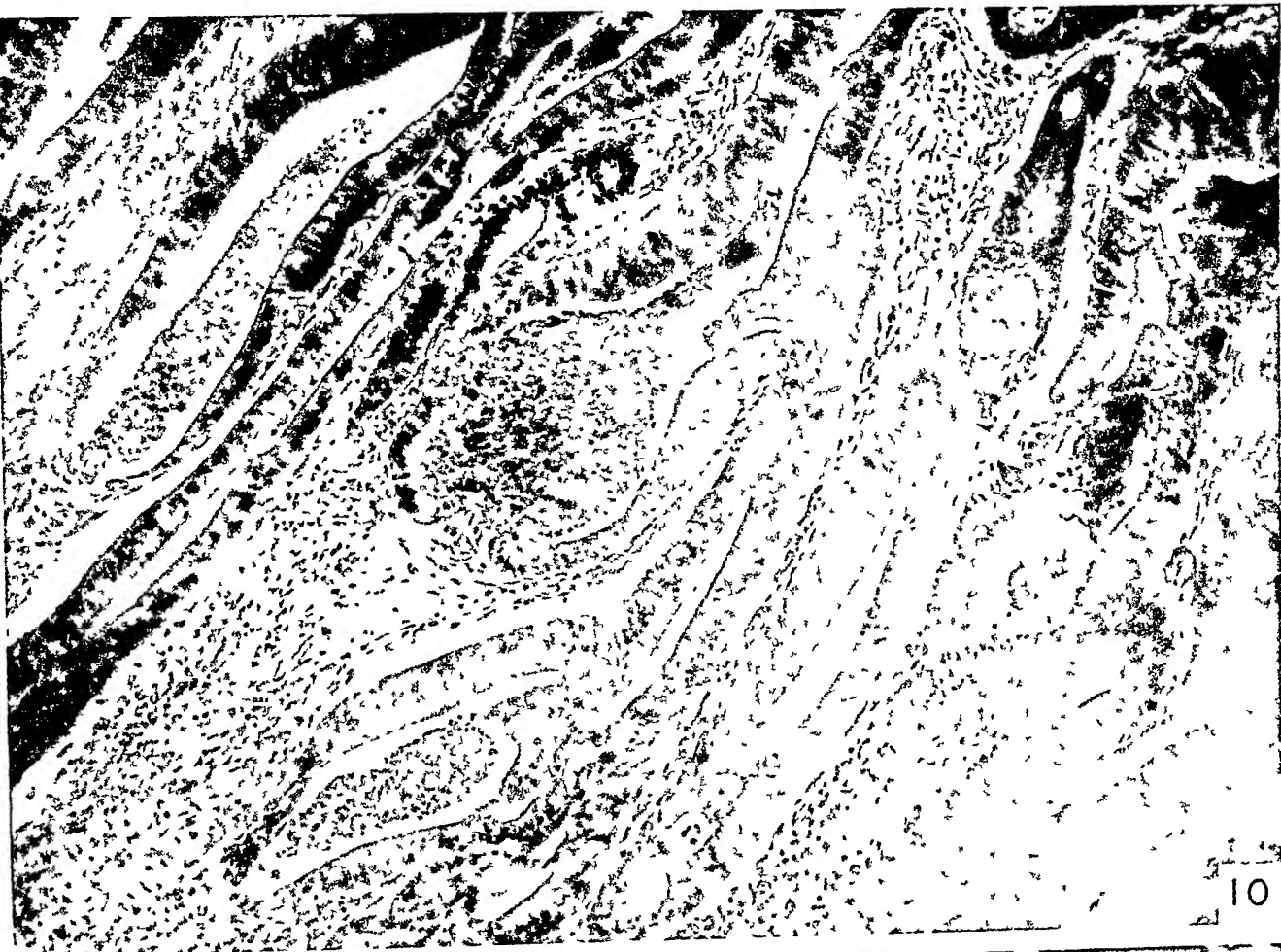


PLATE 163

FIG. 10. Duct-like structures in group III, B. Hematoxylin and eosin stain. $\times 125$.

FIG. 11. Single layer of cylindric cells lining duct-like structure. Squame-like material in lumen. Regaud's iron hematoxylin stain. $\times 400$.

FIG. 12. Pseudo-stratification and stratification of cylindric epithelium. Hematoxylin and eosin stain. $\times 400$.



THE PANCREAS IN UREMIA: A HISTOPATHOLOGIC STUDY *

ARCHIE H. BAGGENSTOSS, M.D.

(From the Section on Pathologic Anatomy, Mayo Clinic, Rochester, Minn.)

In the course of routine examination of histologic sections of the pancreas at necropsy a remarkable degree of dilatation of the acini, flattening of the lining epithelial cells, and inspissation of the secretion was observed in a number of cases of uremia (Fig. 1). Because of the similarity of the histologic appearance of the pancreas in these cases to that observed in so-called fibrocystic disease of the pancreas of children,¹⁻³ it was thought that an investigation of the lesion might be worth while. It was hoped that such a study might give some clues as to the pathogenesis of the similar lesion in fibrocystic disease. With this in mind the following investigation was carried out.

MATERIAL AND METHODS

The histologic appearance of the pancreas was studied in 85 consecutive cases of chronic glomerulonephritis with uremia, in 85 consecutive cases of hypertension (nephrosclerosis) with uremia, and in 100 consecutive cases in which uremia resulted from either hydronephrosis, pyelonephritis, or extrarenal factors. All cases in which the pancreas was the seat of an inflammatory or neoplastic process or in which there was evidence of obstruction of the large pancreatic ducts were excluded from this study. Sections of the pancreas were fixed in formalin, in Zenker's fluid, and in cold acetone. The following stains were used: Hematoxylin and eosin, Congo red, Mallory-Heidenhain's (azocarmine), Mallory's phosphotungstic acid hematoxylin, Weigert's fibrin, mucicarmine, Feulgen's⁴ stain for nuclear material, and Gomori's reticulum stain.

The control series consisted of 200 cases in a similar age group in which uremia was not a contributory cause of death.

The lesions were classified as severe, moderate, or mild. If one-half or more of the acini in each lobule of the pancreas were dilated, the lesion was designated as severe; if many, but less than one-half, of the acini in each lobule were dilated, the lesion was considered as moderate; if only a few of the acini in each lobule were dilated, the lesion was classified as mild.

RESULTS

The histologic appearance of the lesion was the same regardless of the cause of the uremia. The lesion, when present, was found in all parts of the pancreas in approximately the same stage of development.

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Sometimes it was slightly more extensive in the tail, but more often it was slightly more pronounced in the head of the gland.

Although the classification did not take into account the extent of the dilatation of the individual acini, it was found that in general the degree of dilatation varied directly with the number of acini involved. In other words, dilatation of the acini was generally greatest in those cases in which the numbers of acini involved were greatest.

The dilated acini were distributed diffusely throughout each lobule and were not concentrated in any particular portion. The severe form of the lesion is represented by Figures 1, 2A and B, and 3A and B. The dilated acini contained an acidophilic stringy material which appeared to be inspissated secretion and occasionally was laminated. Desquamated cells and nuclei occasionally made up a portion of the material, and leukocytes were present rarely. The substance was stained pink by hematoxylin and eosin, blue by the Mallory-Heidenhain stain, tan by the Mallory phosphotungstic acid hematoxylin stain, and blue by the Weigert fibrin stain. Stains for mucin gave negative results.

Associated with dilatation of the acini was a variable degree of flattening of the lining cells. Normally, these cells are pyramidal with the apex toward the lumen. In the dilated acini the cells were cuboidal and occasionally so markedly flattened that one could see only a thin rim of cytoplasm flattened against the basement membrane. The nuclei which normally are spherical were often oval or elongated with the long axis parallel to the basement membrane. The nuclei frequently were pyknotic. Less frequently, karyolysis and karyorrhexis were observed. In addition to the changes in the shape of the cells, there was also a loss of the normal staining reaction. This change could usually be observed best when there was only a mild degree of dilatation. With the hematoxylin and eosin stain the basal portion of the cell, which normally is purple, had an eosinophilic appearance. The zymogen granules in the supranuclear portion of the cell were absent (Fig. 2B). Instead of the two usually distinct zones, the entire cytoplasm had a homogeneous eosinophilic, almost hyaline appearance. From a study of those cases in which the pathologic process was mild and presumably in an early stage of development, it appeared that cytologic changes often, but not always, antedated acinar dilatation.

In some cases in which the degree of dilatation of the acini of the pancreas was severe, there was complete disappearance of a cell or two from the lining. Rarely an entire acinus was destroyed and replaced by fibroblasts and reticulum fibers. This was not a prominent feature in any of the cases, however.

In addition to dilatation of the acini, the tiny ductules were fre-

quently dilated also. Medium-sized and large ducts were not involved in the process, however. Squamation (metaplasia) of the epithelium of the ducts was infrequently observed and, when present, it occurred as an isolated lesion. It was present in 9 cases in which dilatation of the acini was observed and in 4 cases in which there was no dilatation of the acini. In no instance had it led to anatomic obstruction of a duct.

Although vascular lesions, particularly arteriosclerosis, and their sequelae, such as thrombosis, focal infarcts, or regions of atrophy, were frequently observed, they will not be considered in this study except to point out that such lesions were as frequently present in cases in which dilatation of the pancreatic acini did not occur as in cases in which dilatation did occur. Mild to moderate interlobular fibrosis and fatty replacement of the pancreas were frequently observed in both groups of cases, particularly in those subjects more than 60 years of age.

Chronic Glomerulonephritis with Uremia

Examples of the pancreatic lesion in uremia resulting from chronic glomerulonephritis are shown in Figures 1A and 1B. The incidence and the degree of dilatation of the acini in this group of cases are shown in Table I. In the group of 33 cases in which the pancreatic lesion

occurred, there were 20 males. The average (mean) age was 33 years, the mean value for blood urea was 344 mg. per 100 cc., and pericarditis was present in 17 cases (52 per cent). Nausea and vomiting were among the symptoms in 24 cases (73 per cent).

In the group of 52 cases in which the pancreatic lesion did not occur, there were 31 males. The mean

age was 34 years, the mean value for blood urea was 253 mg. per 100 cc., and pericarditis occurred in 11 cases (21 per cent). Nausea and vomiting were among the symptoms in 24 cases (46 per cent).

Hypertension (Nephrosclerosis) with Uremia

Examples of the pancreatic lesion in uremia resulting from nephrosclerosis are shown in Figure 2. The incidence and the degree of dilatation of the acini in this group of cases are shown in Table II. Among the 36 cases in which the lesion occurred, there were 25 males. The mean age was 47 years, the mean value for blood urea was 267 mg. per 100 cc., and pericarditis was present in 15 cases (42 per cent).

TABLE I
*Pancreas in Uremia: 85 Cases of Chronic
Glomerulonephritis with Uremia*

Dilatation of acini of pancreas	Cases	Per cent
Absent	52	61
Present	33	39
Mild	19	58
Moderate	9	27
Severe	5	15

TABLE II

Pancreas in Uremia: 85 Cases of Hypertension (Nephrosclerosis) with Uremia

Dilatation of acini of pancreas	Cases	Per cent
Absent	49	58
Present	36	42
Mild	22	61
Moderate	12	33
Severe	2	6

Nausea and vomiting were listed among the symptoms in 22 cases (61 per cent).

Among the 49 cases in this group in which the lesion did not occur, there were 40 males. The mean age was 49 years, the mean value for blood urea was 220 mg. per 100 cc., and pericarditis was present in 12 cases (24 per cent).

Nausea and vomiting were among the symptoms in 25 cases (51 per cent).

Uremia Resulting from Miscellaneous Causes

Examples of the pancreatic lesion in uremia resulting from hydro-nephrosis, pyelonephritis, and extrarenal factors are shown in Figures 1C and D, and 3. The incidence and the degree of dilatation of the acini in this group of cases are shown in Table III. Among the 52 cases in which the pancreatic lesion occurred, there were 14 cases of obstructive nephropathy (cases of tubular, pelvic, ureteral, vesical, prostatic, and urethral obstruction), 18 cases of pyelonephritis, and 20 cases in which extrarenal factors were responsible for the uremia. Among these cases there were 39 males and 13 females. The mean age was 58 years, the mean value for blood urea was 224 mg. per 100 cc., and pericarditis was present in 5 cases (10 per cent). Nausea and vomiting were listed among the symptoms in 14 cases (27 per cent).

Among the 48 cases in this group in which the pancreatic lesion did not occur, there were 15 cases of obstructive nephropathy, 15 cases of pyelonephritis, 11 cases of uremia resulting from extrarenal factors, 5 cases of toxic tubular degeneration, and 2 cases of infarcts of the kidneys. There were 32 males and 16 females. The mean age was 49 years, the mean value for blood urea was 227 mg. per 100 cc., and pericarditis was present in 4 cases (8 per cent). Nausea and vomiting were listed among the symptoms in 12 cases (25 per cent).

TABLE III

Pancreas in Uremia: 100 Cases of Uremia Due to Miscellaneous Causes

Dilatation of acini of pancreas	Cases	Per cent
Absent	48	48
Present	52	52
Mild	28	54
Moderate	16	31
Severe	8	15

Control Series

The incidence (20 per cent) and the degree of dilatation of the acini in the control series of 200 cases are shown in Table IV. It should

be emphasized that the incidence of the pancreatic lesion in uremia is significantly greater than in the controls. In none of the cases was a severe degree of dilatation of the acini revealed. The cytologic alterations and the appearance of the inspissated secretion, however, were identical with those observed in the pancreas of uremia. The

cases in the control series in which dilatation of the pancreatic acini occurred were analyzed as to the causes of death; the results are shown in Table V. It is probably significant that intestinal obstruction was the most common cause of death in this group. Among the 40 cases in which the lesion occurred, nausea and vomiting were listed among the symptoms in 14 (35 per cent). Pericarditis was absent in all of these cases. Although uremia was not considered a contributory cause of death in any of these cases, azotemia of variable degree had been present in 10 of the 40 cases.

TABLE V
Pancreas in Uremia: Cause of Death in 40 Controls in Which Dilatation of the Acini of the Pancreas Occurred

Cause of death	Cases
Intestinal obstruction	11
Cancer	7
Infections	7
Congestive heart failure	6
Intracranial lesions	6
Chronic ulcerative colitis	3
Total	40

the pathogenesis of these lesions. Although obstruction of the ducts may give rise to the histologic picture described, it is obvious that the obstruction in these cases in which the lesion was generalized would of necessity be at the outlet of the main pancreatic duct. No such obstruction was found and, furthermore, there was no evidence of dilatation of the large and medium-sized ducts. Metaplasia of the ductal epithelium could not be considered as a cause since it was infrequently found both in cases in which the lesion did and in which it did not occur. Furthermore, if metaplasia had produced obstruction, the dilatation of acini would have been localized to the distribution of the involved duct and not generalized. Whether obstruction of acini and ductules by inspissated secretion plays a rôle in pathogenesis remains to be considered.

TABLE IV
Pancreas in Uremia: 200 Controls Without Uremia

Dilatation of acini of pancreas	Cases	Per cent
Absent	160	80
Present	40	20
Mild	33	82
Moderate	7	18
Severe	0	0

Among the cases in which the lesion did not occur, pericarditis was present in 7 (4 per cent). Nausea and vomiting were listed among the symptoms in 22 cases (14 per cent).

COMMENT

Anatomic and histologic study of the pancreas gave no clue as to

Interest in the lesion under discussion was aroused because of the similarity of the histologic appearance to that observed in fibrocystic disease of the pancreas. However, the lesions are not identical. In the early stages of fibrocystic disease, dilatation of the acini may not be any greater than that observed in these cases of uremia.⁵ However, in the former there is dilatation of the small ducts as well as of the acini, there is always more acinar destruction, and there is decidedly more interstitial proliferation of connective tissue.⁵ It may be postulated that if the patients with uremia survived long enough, the histologic appearance of the pancreatic lesion would ultimately be identical with that of fibrocystic disease. Although this possibility must be considered, it cannot be decided with the material available for study.

Wallace and Ashworth⁶ observed what appears to be a similar lesion in 45.5 per cent of 200 unselected cases. The changes in the acini described by them occurred as either a focal or a diffuse process, whereas in this study the lesion, when present, was always diffuse. In their study, 56 per cent of patients 50 years of age or over had dilatation of acini, while 38.3 per cent of those below 50 years presented this change. Andrew⁷ described locule formation in senile rats and human beings. In photomicrographs, the lesions he described in man do not resemble the acinar dilatation with which this study is concerned. The lesions he described are usually interpreted as foci of atrophy and fat replacement.

It is apparent from the data of the present study that neither age, sex, nor the degree of azotemia played a significant rôle in the pathogenesis of the lesion. From the limited number of cases in which accurate information was available, it appeared that the duration of the uremic state was of no importance in the production of the lesion.

The higher incidence of pericarditis in the cases of glomerulonephritis and nephrosclerosis in which the pancreatic lesion occurred is interesting, but it is difficult to interpret because the factors responsible for uremic pericarditis are not known. It is to be noted also that in the group of cases in which uremia was the result of miscellaneous causes, no such variation in the incidence of pericarditis was found.

It is clear from an examination of the control series that the pancreatic lesion occurs in a variety of conditions other than uremia. The high incidence of intestinal obstruction in the control series suggested that dehydration might be a factor. Dehydration is frequently a complication of renal insufficiency of any type. If dehydration could result in inspissation of pancreatic secretion, it might result in a generalized obstruction of the acini and ductules. In this connection it may be significant that the incidence of vomiting was greater in the cases of

glomerulonephritis and nephrosclerosis in which the pancreatic lesion occurred than in those in which the lesion did not occur. Vomiting would be expected to augment the dehydration in these cases. However, in the group of cases in which uremia resulted from miscellaneous causes there was no significantly greater incidence of vomiting in the cases in which the pancreatic lesion occurred.

In addition to dehydration, other factors which alter the physical and chemical characteristics of pancreatic secretion must be considered. It is a generally accepted fact that nervous (parasympathetic) stimulation of the pancreatic secretion results in the production of a viscid juice rich in enzymes and proteins. After such stimulation the acinar cells shrink and the zymogen granules disappear. Hormonal (secretin) stimulation, on the other hand, produces a more watery juice, poor in enzymes and proteins, without marked histologic changes in the acinar cells. According to Farber,⁸ the lesion observed in fibrocystic disease of the pancreas can be reproduced in kittens in all important respects by the injection of parasympathomimetic drugs. For this reason, the question arises as to whether the state of uremia is accompanied by excessive parasympathetic stimulation. No satisfactory answer to this question has as yet been obtained. The fact that bradycardia is not a feature of the uremic state suggests that vagus stimulation is not significant.

Another question also arises: Is there an inhibition of hormonal (secretin) stimulation of the pancreas in uremia and intestinal obstruction? Normally, the secretin effect may be elicited by the presence of acids, meat extracts, protein derivatives, fats, fatty acids, soaps, and water in the intestine. If these substances were excluded from the intestinal tract, it is conceivable that the normal release of secretin might not occur and hormonal stimulation of the pancreas might be inhibited. Severe repeated vomiting might bring about such conditions. Repeated vomiting is a feature of both uremia and intestinal obstruction.

If the hormonal (secretin) stimulation of the pancreas were inhibited, the only stimuli to secretion would be nervous (parasympathetic). Such an unbalanced stimulation of the pancreas would result in a viscid juice which, if accompanied by a relative state of dehydration, might become inspissated and obstruct the ductules and acini. Stimulation by secretin has been described as causing a flow of alkaline fluid which serves to flush the alveoli, to thin the juice rich in organic material, and to sweep it along the ducts (Best and Taylor⁹). Because of these considerations, it is suggested that the pancreatic lesion described in this study is the result of an interference with the release and normal

action of secretin brought about by excessive vomiting. If this concept of the pathogenesis of the pancreatic lesion is correct, then one would expect to find it frequently in cases of obstruction of the stomach and small intestine. Such cases are now being investigated.

Does this concept of the pathogenesis of the pancreatic lesion give any clues as to the factors responsible for fibrocystic disease of the pancreas? There is no evidence that excessive parasympathetic stimulation occurs in this disease. Excessive and repeated vomiting likewise is not a feature of this condition. One can, however, postulate that there is a congenital absence of secretin in the intestinal tract or that there is some defect in the mechanism of its release. In support of this viewpoint is the fact that the pancreatic juice is very thick and inspissated and that inspissation of bile and of the secretion of the intestinal glands also occurs (Farber³). There is some evidence that, normally, secretin stimulates the secretion of bile and probably the succus entericus (Best and Taylor,⁹ Ågren¹⁰). It has even been called an intestinal diuretic (Ågren). If stimulation by secretin were absent, the obstruction of the small bile ducts and intestinal glands in these cases also might be explained as the result of the production of an abnormally thick inspissated secretion.

It is obvious that more information on secretin is needed. This is particularly true in regard to its presence, mode of formation, and the mechanism of its release in the human being. When this information is available, it may be possible to obtain a better understanding of the pathogenesis of the pancreatic lesion in uremia and of fibrocystic disease of the pancreas.

SUMMARY AND CONCLUSIONS

Histologic examination of the pancreas at necropsy in subjects in which death had resulted from uremia frequently revealed a remarkable degree of dilatation of the acini, flattening of the lining epithelial cells, and inspissation of secretion. The lesion was found in 33 (39 per cent) of 85 cases of uremia resulting from chronic glomerulonephritis, in 36 (42 per cent) of 85 cases of uremia resulting from hypertension (nephrosclerosis), and in 52 (52 per cent) of 100 cases of uremia resulting from miscellaneous causes. Neither age, sex, nor degree or duration of azotemia appeared to play a significant rôle in the production of the lesion. In the control series of 200 cases in which uremia did not occur, the lesion was present in mild or moderate degree in 40 cases (20 per cent). The most common cause of death in the control series was intestinal obstruction. It is suggested that dehydration plus an interference with the release and normal action of secretin

brought about by excessive vomiting are responsible for the lesion. It is also suggested that either a congenital lack of secretin or some defect in the mechanism of its release may be responsible for fibrocystic disease of the pancreas.

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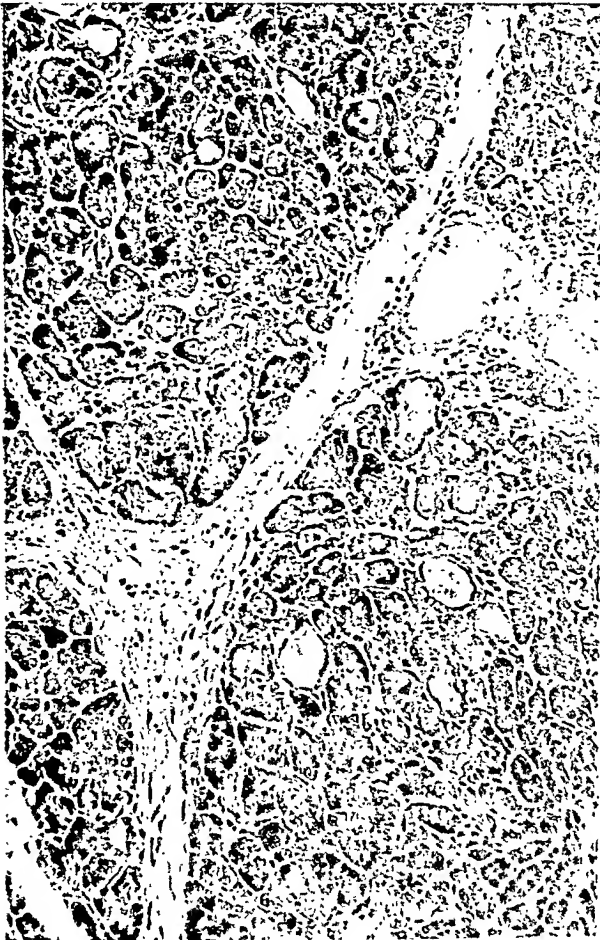
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DESCRIPTION OF PLATES

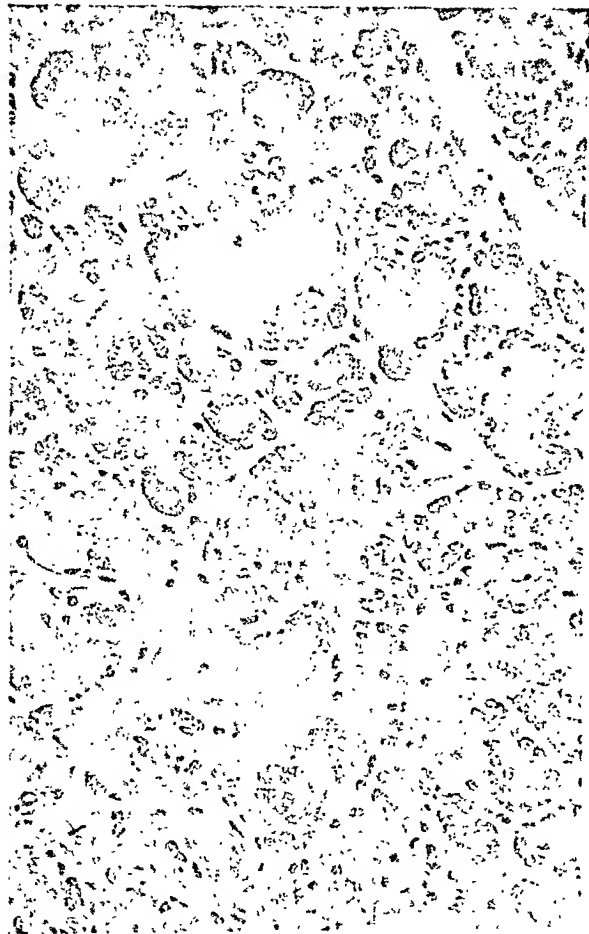
PLATE 164

FIG. 1. Pancreas in uremia. A. In a case of glomerulonephritis; approximately one-half of the acini are dilated. Hematoxylin and eosin stain. $\times 115$. B. Same as A. Hematoxylin and eosin stain. $\times 275$. C. Pancreas in a case of pyelonephritis with uremia, showing dilatation of acini, atrophy of lining epithelium, and inspissated secretion. Hematoxylin and eosin stain. $\times 115$. D. Same as C. Hematoxylin and eosin stain. $\times 265$.

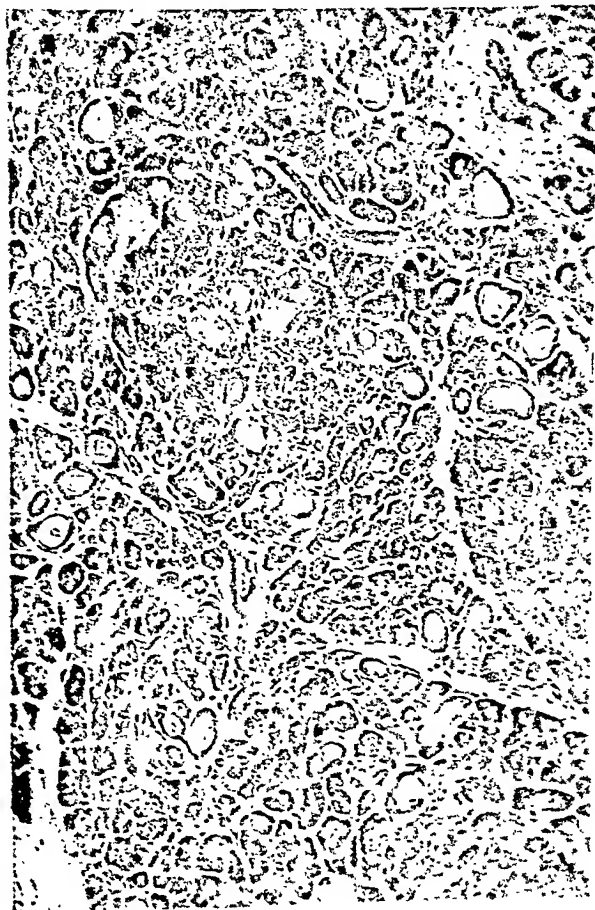
1A



1B



1C



1D

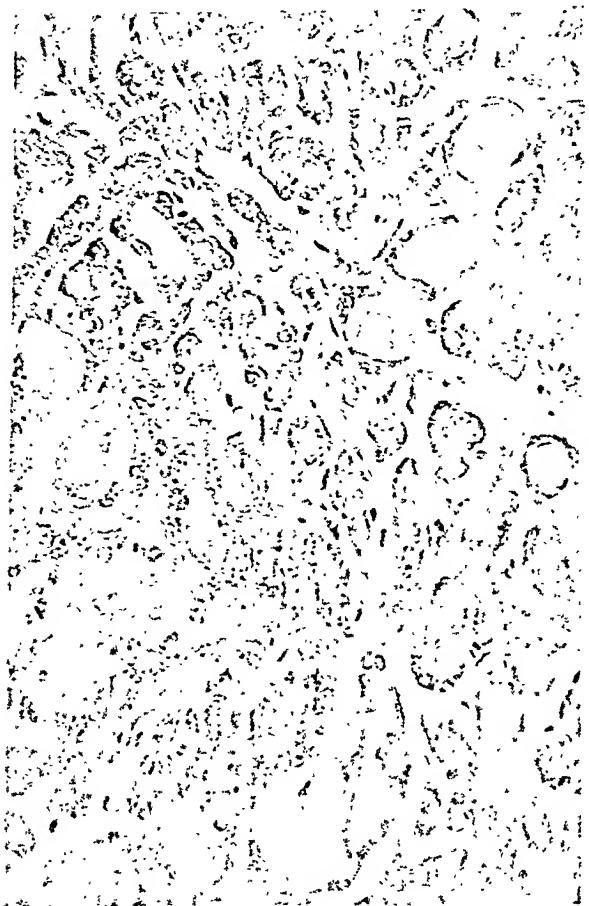
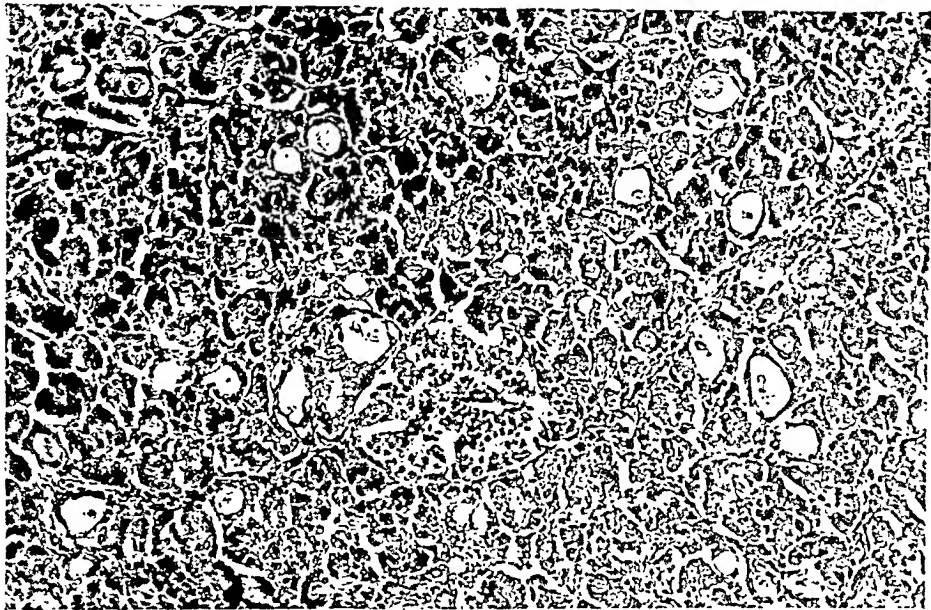


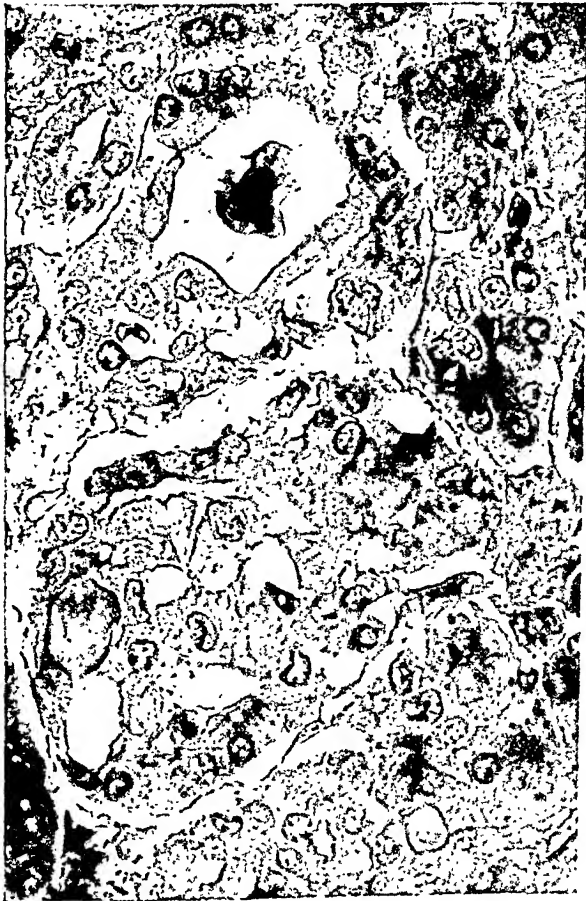
PLATE 165

FIG. 2. Pancreas in uremia associated with hypertension (nephrosclerosis). A. Severe form of the lesion. Hematoxylin and eosin stain. $\times 115$. B. Absence of zymogen granules in epithelial cells of dilated acini. Mallory's aniline blue stain. $\times 525$. C. A moderate number of acini are dilated. Hematoxylin and eosin stain. $\times 115$.

2A



2B



2C

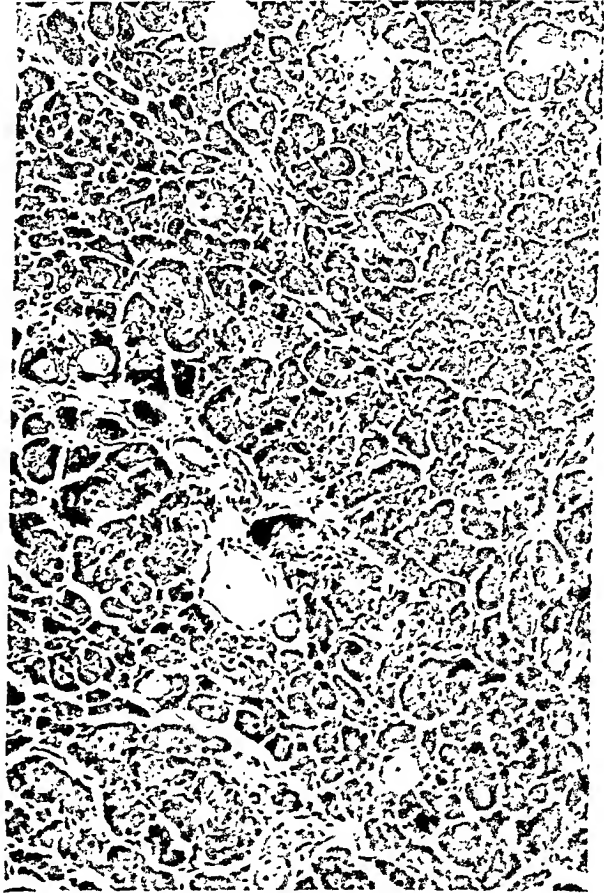
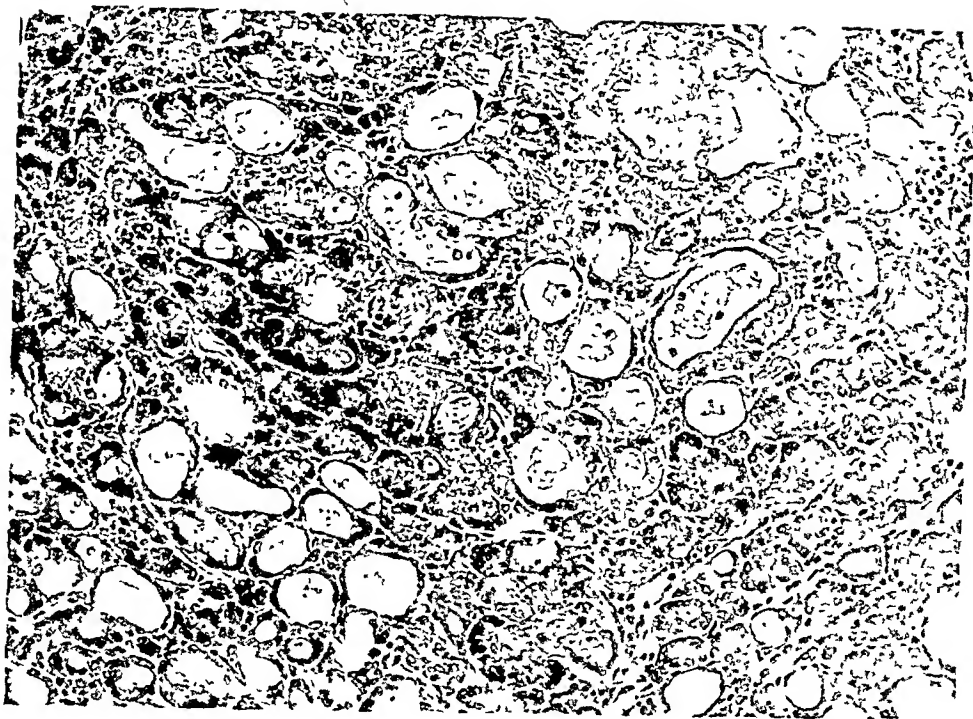


PLATE 166

FIG. 3. Pancreas in uremia from miscellaneous causes. A. Pyelonephritis with uremia; a severe lesion. Hematoxylin and eosin stain. $\times 145$. B. Hydro-nephrosis with uremia; a severe form of the lesion. Hematoxylin and eosin stain. $\times 265$. C. Extrarenal uremia; a moderate number of acini are dilated. Hematoxylin and eosin stain. $\times 115$.

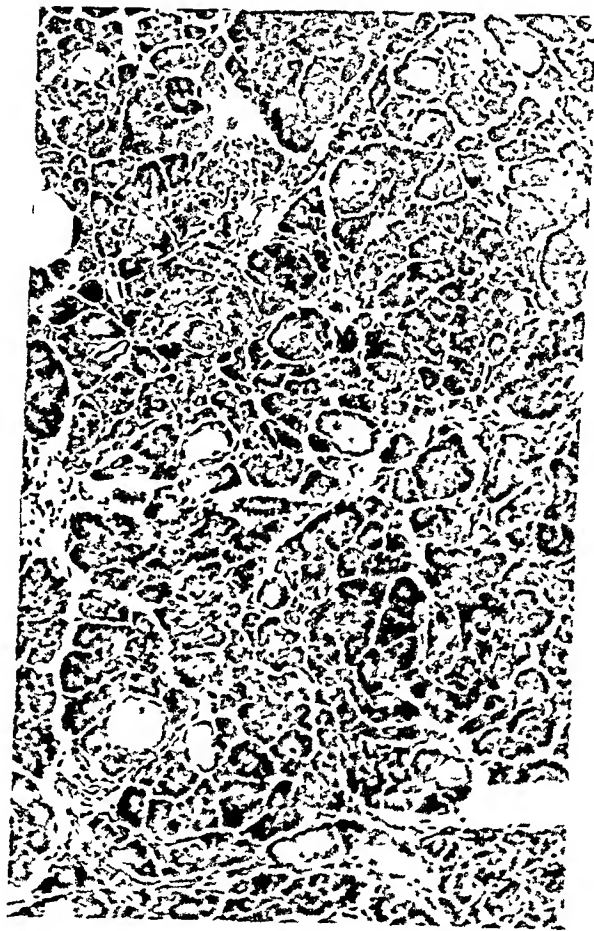
3A



3B



3C



Baggenstoss

The Pancreas in Uremia

SKELETAL EFFECTS OF ESTROGENIC HORMONE IN GROWING VITAMIN C-DEPLETED GUINEA-PIGS *

MARTIN SILBERBERG, M.D., and RUTH SILBERBERG, M.D.

(From the Snodgrass Laboratory of Pathology, City Hospital, and the Barnard Free Skin and Cancer Hospital, St. Louis, Mo.)

The action of estrogenic hormones on the mesenchymal tissues is not yet fully understood. The response varies with species, strain, and sex, and with the dose administered.^{1,2} In the growing animal, the main changes consist of hyalinization of connective tissue, vascular walls, and of cartilage associated with increased ossification. This reaction is marked in certain strains of mice and in some birds; it is less pronounced in the growing rat and guinea-pig.¹⁻⁵ Inasmuch as some skeletal effects of estrogenic hormone were attributed partly to changes in the connective tissue, it was thought of interest to alter the state of the interstitial substance by depleting animals of vitamin C, and then study the effect of an estrogen. It was hoped, thus, to gain further insight into the mechanism by which the female sex hormone affects the skeleton.

MATERIAL AND METHODS

Twenty-six guinea-pigs weighing 180 to 200 gm. were divided into four groups. *First Series:* Eight animals (normal controls) received a standard diet of purina rabbit chow meal *ad libitum*. This diet is a mixture of checkers with grain, molasses, and chopped alfalfa, and contains the following:

Protein		15.32 per cent
Fat		3.27 " "
Fiber	} Carbohy-	{ 14.88 " "
N free extract		
Ash		5.89 " "
Ca		0.84 " "
P		0.32 " "
Carotene		11.0 parts per million
Thiamine		5.09 " " "
Riboflavin		3.74 " " "
Niacin		29.7 " " "

Vitamin C was supplied in the form of fresh lettuce, fed daily. Two animals were sacrificed after 2, 3, 4, and 5 weeks. *Second Series:* Four animals were given lettuce and the standard diet, and were injected subcutaneously with 0.17 mg. of alpha-estradiol benzoate in sesame oil three times weekly.[†] One animal each was killed after 2, 3,

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4, and 5 weeks. *Third Series:* Six guinea-pigs were fed purina rabbit chow meal without additional lettuce. Two were sacrificed after 2 and 3 weeks; of the remainder, one died during the fourth week; the last had to be killed at the end of the fourth week. *Fourth Series:* Eight guinea-pigs were kept on the vitamin C-free diet and were injected three times weekly with 0.17 mg. of alpha-estradiol benzoate. Two animals were killed after 2, 3 and 4 weeks. Towards the end of the fifth week, the 2 remaining animals appeared very ill and were then sacrificed.

At necropsy, the tibiae, femora, several lower ribs, vertebrae, and the whole heads were removed. The tibiae were measured with

TABLE I
Mean Initial and Final Weights

Experiment	Initial	2 weeks	3 weeks	4 weeks	5 weeks
	gm.	gm.	gm.	gm.	gm.
Normal	189	291	306	317	398
Estrogen	182	255	351	362	371
Avitaminosis C	188	193	191	127	
Avitaminosis C and estrogen	183	196	283	208	212

calipers. The bones were fixed in 10 per cent formalin, and all except the heads decalcified in 5 per cent nitric acid; sections were prepared and stained with hematoxylin and eosin.

GROSS OBSERVATIONS

The mean initial and final weights are presented in Table I. The normal and estrogen-treated guinea-pigs gained weight steadily during the period of observation. The vitamin C-depleted animals failed to gain weight and showed a sudden loss of weight during the fourth week. The vitamin C-depleted animals treated with estrogen gained weight during the first 3 weeks, although less than those receiving alpha-estradiol benzoate and fed the complete diet. During the fourth week, however, they likewise lost considerable weight, although their weight was still much greater than that of the vitamin C-depleted animals not receiving the estrogen.

In animals fed the vitamin C-free diet, periosteal hemorrhages in the long bones and joints, and especially in the lower ribs, appeared after 2 weeks and they became more extensive with increasing duration of the experiment. After 3 weeks, large bead-like swellings at the chondro-osseous junctions of the ribs were noted. The bones were thin and fragile, whereas those of the animals receiving estradiol benzoate were thicker and harder than the normal bones. In the guinea-pigs fed the vitamin C-free diet and treated with the estrogen, rosaries were absent

except for some slight swelling of two ribs in one of 8 animals. Hemorrhages in the periosteal tissues and joints were not seen before the end of the fourth week, and then they were less extensive than in the noninjected vitamin C-depleted animals.

As seen from Table II, the lengths of the tibiae of the normal animals increased steadily in the course of 5 weeks. The tibiae of the guinea-pigs receiving estrogen grew more slowly during the first 4 weeks, but developed a growth spurt during the fifth week. In animals kept on the vitamin C-free diet, an insignificant increase in the length

TABLE II
Mean Lengths of the Tibiae

Experiment ²	Initial	2 weeks	3 weeks	4 weeks	5 weeks
	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
Normal	3.28*	3.45	3.48	3.87	3.93
Estrogen		3.29	3.45	3.49	3.79
Avitaminosis C		3.37	3.61	3.32	
Avitaminosis C and estrogen		3.29	3.46	3.47	3.45

* These values represent means of 4 normal animals weighing 180 gm., and used in former experiments.

of the tibiae was observed during the first 3 weeks; after 4 weeks, the tibiae were shorter than at any earlier period which might be incidental but which might be due also to a collapse of the metaphysis. In vitamin C-depleted guinea-pigs receiving estrogen, the lengths of the tibiae during the first 3 weeks closely paralleled those seen in normally fed animals injected with the hormone. However, a measurable growth spurt occurring in the latter during the fifth week did not take place in the vitamin C-depleted guinea-pigs treated with estrogenic hormone.

HISTOLOGIC EXAMINATION

First Series: Untreated Control Animals

Epiphyses. After 2 or 3 weeks of observation, the epiphyseal plates at the upper end of the tibia were about 350 μ wide and showed a regular configuration (Fig. 1). The cartilage cell rows were separated from each other by thin layers of chondromucoid ground substance. A single column was composed of 10 to 12 proliferating and 4 hypertrophic cartilage cells. After 5 weeks, the growth zone measured 330 μ in height. Moderate amounts of matrix surrounded the individual cell rows which contained 9 columnar and 3 or 4 hypertrophic cells. The three layers of the articular cartilage were distinct, and the ossification of the hypertrophic zone was progressing. The chondro-osseous junction of the ribs and the epiphyseal plates of the femur and vertebrae showed the usual architecture.

Diaphyses. Numerous capillaries eroded the hypertrophic cartilage cells in the metaphysis of long bones, ribs, and vertebrae. In a standard area 1.5 mm. in diameter, 12 to 14 spicules were counted, and after 5 weeks their number had increased to 16 to 18. The trabeculae were slender at the earlier age, but increased in thickness during the 5 weeks of observation. They were covered by a continuous layer of large osteoblasts (Fig. 5). The bone marrow was vascular and cellular. The shaft was composed of medium-sized osteocytes and abundant bony matrix (Fig. 9). The vascular canals were narrow, and the enclosed blood vessels were surrounded by a thin layer of loose connective tissue. The endosteal osteoblasts were numerous and large. The periosteum was thick. Osteoblasts and osteoclasts were present; the latter were situated in grooves of the compacta.

Second Series: Animals Treated with Alpha-Estradiol Benzoate

Epiphyses. After 2 weeks of injections, the growth zones were narrowed to 260 μ . The cartilage cells were smaller and less numerous than in the normal controls, and the matrix was intensely hyalinized and calcified. In the animals receiving 4.99 mg. of the hormone over a period of 3 weeks, a further decrease in the width of the growth zones to 170 μ had occurred (Fig. 2). An individual cell row was composed of 5 or 6 columnar and 3 to 5 small cells of hypertrophic type. The cartilaginous ground substance was heavily calcified, and here and there whole cartilage cell columns had been replaced by thick plugs of sclerosed ground substance (Fig. 6). The articular cartilage cells were smaller than is normal. At the chondro-osseous junction of the ribs and in the femur and vertebrae the growth processes had likewise markedly declined, and regressive changes were accentuated. After 5 weeks, the epiphyseal plate became wider again, the cartilage cells were enlarged, and calcification was less marked.

Diaphyses. After 2 weeks, the metaphyseal capillaries were inconspicuous, poorly filled and accompanied by fibrous tissue. The trabeculae, particularly in their proximal parts, were thicker than ordinarily. This was due chiefly to the presence in their centers of large, unopened cartilage cells still surrounded by some cartilaginous matrix. Along these spicules, a fair number of osteoblasts was noted. The amount of bone present was increased about 25 per cent over normal. The endosteal osteoblasts were delimited from the bone marrow by a layer of fibrillar tissue. The shaft was thick. The haversian canals were narrow and contained poorly filled vessels surrounded by increased amounts of connective tissue. The periosteum was likewise thick and contained much collagen. Near the metaphysis, some osteoclasts were

found, but farther down along the shaft they were scanty or absent. After 3 weeks of injections (Fig. 6), the metaphyseal spicules still contained preserved cartilage cells; they were thick and showed some transverse linking. The peritrabecular connective tissue had increased in amount and the osteoblasts were smaller than is usual. In the distal metaphysis the trabeculae were considerably coarser than in the normal control animal. Conditions in the shaft (Fig. 10) were similar: The vascularization was further decreased, and the bone was solid. Periosteal ossification was advanced, osteoclasts were rare; the shaft had a smooth surface. The periosteum was closely attached to the compact bone, it was dense and contained much collagen; between the fibers, few fibrocytes were seen. After 4 or 5 weeks, the vascularization of the shaft and metaphysis had increased. Numerous large osteoblasts reappeared; the connective tissue was looser than at the earlier experimental stages. Owing to increased resorption of bone, a fibro-osseous network was formed. The vessels perforating the shaft were engorged, the periosteum was looser and contained again more osteoclasts than earlier.

Third Series: Vitamin C-Depleted Animals

Epiphyses. In guinea-pigs kept on the vitamin C-deficient diet for 2 weeks, the growth zones showed the usual width and structure. The proliferation of the columnar cartilage cells and their conversion into hypertrophic cells were not appreciably affected. There may have been a slight decrease in these processes. The cartilaginous ground substance, however, was loose and swollen. After 3 weeks and later, the epiphyseal plate at the upper end of the tibia measured about 325 μ in height and showed an irregular pattern (Fig. 3) owing to the marked swelling of the matrix. The number of cells in an individual column was not changed materially; however, the columnar cartilage cells failed to undergo hypertrophy. Instead of the usual calcium deposits, there appeared in the most distal layer of the cartilage patches of deeply eosinophilic material bearing a certain resemblance to the fibrinoid substance found in the metaphysis. Here and there, capillaries of the bone marrow began to invade the softened epiphyseal cartilage, but many cell capsules were left unopened. The cartilage cells of the joints were decreased in number and size, and there was likewise pronounced swelling of the intercellular matrix. Calcification and ossification of the cartilage were strikingly decreased. The findings at the growth zones of the other bones were similar to those in the tibia. At the chondro-osseous junction of the ribs, a mass of hypertrophic, swollen, vacuolated and poorly calcified cartilage was found.

Diaphyses. After 2 weeks, the metaphyseal capillaries were thin-

walled and dilated; the trabeculae, although present in their usual numbers, were long but thinner than ordinarily; the lines of calcium in the centers of the spicules were distinct. Large succulent osteoblasts were in mitosis; they formed several layers and large clusters in the zone underneath the cartilage and between the trabeculae. Some of the latter had broken down, and were converted into structureless eosinophilic material; particularly near the shaft, hemorrhages were noticeable. In the distal metaphysis, the bone marrow was converted into a loose fibrous tissue. The shaft contained large osteocytes with little cementing substance. The vascular canals were enlarged and filled with edematous connective tissue poor in cells, and some rudimentary osteoblasts. The periosteum was vascular and detached from the cortex of the bone, and hemorrhages were seen. The longer the vitamin C-depletion lasted, the more advanced were these lesions. After 3 weeks and later (Fig. 7), only a few small osteoblasts were present in the metaphysis and at the shaft. Few trabeculae were preserved here and they consisted of hardly more than a short calcified spur of cartilaginous matrix with a narrow rim of bony substance. They were surrounded by a few fibrocytes, elements of an edematous connective tissue which had replaced the normal constituents of the metaphysis. There were widespread hemorrhages into this fibrous tissue. Fragments of poorly ossified or noncalcified spicules and fibrinoid eosinophilic material were abundant. The severity of these lesions varied in different bones and decreased in the following order: Proximal part of the tibia, lower ribs, distal end of femur, proximal end of femur and vertebrae. The latter were involved in only one case. The shaft (Fig. 11) was further thinned out, and the enlarged haversian spaces contained edematous fibrillar tissue with engorged blood vessels. The endosteum was represented by a thin layer of fibrocytes; the periosteum was swollen in its outer layers and showed foci of hemorrhages.

*Fourth Series: Vitamin C-Depleted Animals Treated with
Alpha-Estradiol Benzoate*

Epiphyses. Two weeks after the beginning of the experiment, the epiphyseal plates were narrow ($190\ \mu$), but regularly arranged. Hyalinization and calcification were increased over the normal, and the number of cells in a single row had dropped to 5 or 6 columnar and 3 or 4 hypertrophic cells, both cell types being greatly reduced in size. After 3 weeks, the height of the growth zones and the appearances of the cartilage were about the same as at the earlier stages (Fig. 4); the cartilaginous matrix was abundant and denser than ordinarily, but it was less calcified and looser than in the guinea-pigs receiving the

hormone and kept on the complete diet. During the fifth week, owing to a sudden pronounced swelling of the interstitial substance, the epiphyseal disks became wider. In the most distal layer of the cartilage, some eosinophilic hyaline material was present. Conditions thus were comparable to those seen in vitamin C-depleted noninjected animals after 2 or 3 weeks. Ossification of the articular cartilage was likewise diminished. There were, here and there, minute hemorrhages in the periarticular tissue. In 7 of 8 cases, the chondro-osseous junction was regular except for decreased bone formation. In the one animal showing grossly a slight rosary, conditions resembled those seen at the growth zone of the tibia, although they were less pronounced. No significant changes were noted in the vertebrae.

Diaphyses. After 2 weeks, the vascularization of the metaphysis was decreased but less so than in the estrogen-treated animal kept on the complete diet. The vessels were supported by a dense fibrillar stroma. The trabeculae were about as numerous as in normal controls. Many small osteoblasts had accumulated in close approximation to the hypertrophic cartilage cells and along the spicules, and moderate amounts of organic matrix had been deposited. Farther distally, the trabeculae were composed of solid matrix and a core of calcium salts. They were shorter, thinner, and less compact than in the animals receiving estrogen with the complete diet. On the other hand, they contained more osseous matrix than the noninjected vitamin C-depleted controls. The spicules were covered with medium-sized osteoblasts which were, in turn, surrounded by a loose connective tissue. There were no deposits of fibrinoid material nor hemorrhages as commonly found at this stage in noninjected vitamin C-deficient animals. The shaft was of the usual thickness. The endosteal osteoblasts were separated from the bone marrow by a layer of fibrocytes. The haversian canals contained engorged capillaries accompanied by dense stroma with some osteoblasts. The periosteum was dense and contained many fibrocytes, medium-sized osteoblasts, and a few osteoclasts. At the chondro-osseous junction of the ribs and in the vertebrae, the spicules were long, but thinner than in the estrogen-treated animals kept on the complete diet. After 3 weeks (Fig. 8), the metaphyseal capillaries were dilated and much loose supporting stroma was present. The trabeculae were longer than after 2 weeks, showed some interlacing, and were covered by spindle-shaped osteoblasts. They were thinner than in the estrogen-injected animals fed the complete diet, but they were more numerous and thicker than the trabeculae in the vitamin C-depleted animals receiving no hormone. The shaft was fairly thick (Fig. 12). The amount of bone present surpassed that seen in the

vitamin C-deficient, noninjected guinea-pigs. The endosteum was composed of fibrocytes and small osteoblasts. The haversian canals were wider than ordinarily but less numerous, and they were narrower than in the noninjected vitamin C-deficient animals. The periosteum was fibrillar; near the compacta, osteoblasts and osteoclasts could be identified. Again in ribs and vertebrae, the changes were less accentuated than in the tibia; there was in particular no fibrosis, hemorrhages, nor deposition of fibrinoid substance in the former. After 4 weeks, some of the thinner spicules in the metaphysis began to break down, and fragments of bone came to lie in an oblique or transverse direction. Some long spurs of calcified cartilaginous matrix with only a very thin covering of bony substance were seen. In the peripheral areas of the metaphysis, fibrous tissue had replaced the bone and some of the bone marrow. The cortex of the shaft was thinner than at earlier periods. The connective tissue in the enlarged haversian spaces and in the periosteum was edematous. Changes in ribs, femora, and vertebrae were either slight or absent. During the fifth week, the destruction of the metaphysis made rapid progress, and the findings resembled those seen in noninjected animals kept on the C-deficient diet for 3 weeks. The effects of estrogen, however, could still be recognized by the large number and the plumpness of the bony fragments present in the fibrous tissue. Frequently, the fibrinoid material showed the shape and the perpendicular arrangement of spicules and some giant cells were found in the vicinity of these structures. Also the shaft was more solid. The metaphysis of the other bones was likewise better preserved than in any of the vitamin C-depleted animals receiving no hormone.

DISCUSSION

In Table III, the histologic findings in the various experimental and control groups are summarized. As seen from this table, alpha-estradiol benzoate decreased the growth of the epiphyseal cartilage, the vascularization of the metaphysis and shaft, and the resorption of bone, but it intensified hyalinization of cartilage and connective tissue in the long and spongy bones. As observed previously, a reversal of the estrogen effect occurred during the fifth week; it manifested itself in a resumption of growth and resorptive processes. This reversal was considered as due to an adaptation of the organism to the hormone.¹

Vitamin C-depletion altered the proliferation of the cartilage only to a slight degree or not at all, but it caused swelling of cartilage cells and matrix, and increased the resorptive processes besides producing the lesions typical of scurvy.⁶⁻¹⁰

These changes appeared after 2 or 3 weeks of feeding the deficient

TABLE III
Schematic Presentation of the Changes Taking Place in the Skeletal Tissues after Four Weeks of Observation

	Cartilage			Bone (metaphysis and shaft)		Connective tissue (metaphysis and periosteum)	Vessels
	Proliferation	Hypertrophy	Regression	Amount	Resorption		
Estrogen-treated guinea-pigs	Decreased	Decreased	Increased hyalinization and calcification	Increased	Decreased	Increased, dense, hyalinized; osteoblasts small, not increased in number	Thick-walled, vascularization decreased
Vitamin C-depleted guinea-pigs	Slightly decreased	Somewhat decreased	Marked swelling	Markedly decreased	Markedly increased	Loose, edematous, acellular; osteoblasts scarce, replaced by fibrocytes	Thin-walled, congested, vascularization increased, extensive hemorrhages
Estrogen-treated and vitamin C-depleted guinea-pigs	Decreased	Decreased	Some swelling	Somewhat decreased	Somewhat decreased	Somewhat increased, decreased cellularity; osteoblasts fairly numerous, spindle-shaped	Somewhat thin-walled, vascularization somewhat decreased, some hemorrhages

diet and were most advanced in the tibia and lower ribs. The hip bone and the vertebrae, which are less subject to mechanical stress than the former, were less or not at all affected. These findings corroborated the previously established importance of mechanical factors in the production of scorbutic lesions.⁸⁻¹⁰

The effects of alpha-estradiol benzoate in vitamin C-depleted guinea-pigs depended upon the duration of the experiment. During the first 3 weeks, the effects of estrogen predominated, as was indicated by decreased growth processes in the cartilage and by decreased resorption of bone. At the same time, the vitamin deficiency manifested itself by a diminution in the hyalinization of the cartilage, some loosening of the connective tissue, and diminished amounts of bone, as compared with conditions in animals receiving the hormone but kept on the complete diet. After 4 weeks, and coinciding with the resumption of growth and resorptive processes seen in normally fed estrogen-treated guinea-pigs, vitamin C-depletion gained the upper hand, and during the fifth week, the scorbutic lesions progressed rapidly and with great intensity. However, the severe changes of scurvy were limited to the metaphyses of the long bones. The ribs showed only minor lesions, and the vertebrae were hardly involved at all.

The present findings may be considered from two aspects: (1) The development of scurvy as altered by the administration of alpha-estradiol benzoate, and (2) the modification of the estrogen effect by the absence of vitamin C.

Under the influence of the estrogen, growing guinea-pigs fed a vitamin C-free diet survived longer, and the skeletal lesions of scurvy appeared about 2 weeks later than in animals not receiving this hormone; but once lesions had set in, they developed in a precipitous manner. Such an effect might be obtained if the estrogenic hormone exerted a sparing effect on the vitamin C stores of the growing guinea-pig. But there is no real proof that estrogen acts in this way.

A more probable reason for the delay of the scorbutic sequence may be the inhibition of the growth of the cartilage caused by the estrogen. During the growth period, the metaphysis is constantly being remodeled by deposition of new bone and subsequent resorption of the primary spongiosa. In the absence of vitamin C, the formation of bone ceases, and the metaphysis, unable to bear the mechanical stress exerted upon it, breaks down. In the vitamin C-depleted growing guinea-pigs treated with estrogen, growth of cartilage is temporarily arrested and conditions are thus comparable to those in adult scorbutic animals. After cessation of growth, the rapid turnover of metaphyseal tissue has ceased, and the epiphysis is more securely anchored on the metaphysis

than in the growing animal. Under these circumstances, the typical sequence of scorbutic changes does not develop in the metaphysis.¹¹ However, after several weeks of administration of estrogenic hormone in the young guinea-pig, growth is resumed, and then the scorbutic skeletal lesions appear.

The inhibition of the resorption of bone produced by the estrogen may constitute another factor in the modified course of scurvy. In the absence of vitamin C, no new bone is formed and thus the equilibrium between deposition and resorption of osseous tissue is disturbed in favor of the latter. Obviously, the resulting atrophy of bone would be expected to be counteracted by the retardation of resorptive processes caused by the estrogen. Again, the restoration of resorption after the adaptation of the organism to the hormone will bring about a rapid appearance of the skeletal lesions of scurvy.

As to a modification of the skeletal effect of estrogen by vitamin C deficiency, inhibition of growth of cartilage—an estrogen effect—is not affected by the lack of vitamin C. On the other hand, hyalinization and calcification of the cartilage—another estrogen effect—are strikingly counteracted by the marked swelling of the cartilage caused by the vitamin C deficiency. The mode in which estrogenic hormone leads to bone formation is still under discussion. The increase of bone or bone-like tissue seen under the influence of the estrogen is not accompanied nor preceded by a proportionate increase in the number of osteoblasts. Instead of an active osteoblastic layer, a fibrous tissue is frequently found in the immediate vicinity of the excess bone. It seems difficult to understand how the spindle-shaped, apparently inert cells of this fibrous tissue could act as osteoblasts. Therefore, a decrease in the resorption of bone was thought to be at least partly responsible for the presence of increased amounts of bone seen under the influence of the estrogen; ^{1, 4, 12} furthermore, the decreased vascularization of the metaphysis and the deposition of inert hyaline material around the vessels were considered conducive to bone formation.⁵ More recently, in x-ray diffraction studies in estrogen-treated mice, the material deposited adjacent to the pre-existent bone was shown not to be true bone salt—apatite, but more likely calcium carbonate.¹³ This finding may be linked up with the replacement of osteoblasts by fibrous tissue as seen after administration of estrogenic hormone. Connective tissue cells may fail to differentiate into mature osteoblasts and may thus be unable to form true bone, but they may yet to some degree be capable of promoting the precipitation of less complex calcium salts from the serum. This phenomenon might be interpreted as bone formation of a more primitive type.¹⁴ The results of the present investigation sug-

gest that besides connective tissue cells and calcium salts, a third element, namely, an intact interstitial matrix, is a prerequisite for the production of this "primitive bone." After administration of alpha-estradiol benzoate, less bone-like tissue was found in vitamin C-deficient animals than in those fed the complete diet. In view of the established effects of vitamin C on the intercellular matrix, it may be assumed that the inadequacy of the latter accounts for the failure of the estrogen to exert its usual effects on bone.

The effects of the estrogen on the vessel walls are likewise counteracted by ascorbic acid deficiency. Actual hyalinization of the capillary walls or deposition of hyaline material in the perivascular connective tissue as seen in mice and birds is difficult to demonstrate in the guinea-pig. However, in the latter species, the vessel walls are thickened after treatment with alpha-estradiol benzoate. Both the metaphyseal and periosteal blood channels are affected in this manner. The increased thickness of the vessel walls and the associated loss of elasticity are at least partly responsible for a decrease in the resorptive processes. In vitamin C-depleted guinea-pigs injected with estrogen, these vascular changes were not observed, and for this reason resorptive processes could take place in a more normal fashion. The inability of the organism to form cementing substances in the vessel wall in avitaminosis C may account for the absence of this estradiol effect. Again, as in the case of the cartilaginous ground substance, this interference is probably a true antagonistic effect of two factors acting on the same constituent of the intercellular matrix.

SUMMARY

In growing guinea-pigs, the absence of vitamin C modifies the skeletal effects of alpha-estradiol benzoate as follows: The calcification and hyalinization of the ground substance caused by the hormone are counteracted, while the suppression of growth of cartilage produced by estrogenic hormone is not affected. Excessive amounts of bone in the metaphysis found in normally fed animals under the influence of estrogen are absent in avitaminosis C. This is probably due to the inability of the intercellular matrix to be converted into bone, if vitamin C is lacking. Likewise, thickening of the vessel walls seen after treatment with estrogen does not take place in hormone-treated vitamin C-deficient animals. The onset of the scorbutic sequence in the metaphysis of the long bones is retarded and its appearance in ribs and vertebrae is counteracted by the administration of estrogenic hormone to vitamin C-depleted animals.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 167

FIG. 1. Section through the growth zone at the upper end of the tibia of a male guinea-pig, whose weight had increased from 185 to 305 gm. during 3 weeks of observation. The epiphyseal plate and the metaphysis show a regular configuration. $\times 95$.

FIG. 2. Section through the growth zone at the upper end of the tibia of a male guinea-pig which received a total of 150 mg. of alpha-estradiol benzoate over a period of 3 weeks. The weight of the animal had increased from 182 to 351 gm. during the time of observation. The epiphyseal plate is narrowed and heavily calcified, the cartilage cells are smaller, and the trabeculae, particularly in their distal parts, are denser and thicker than in Figure 1. $\times 95$.

FIG. 3. Section through the growth zone at the upper end of the tibia of a male guinea-pig kept on a vitamin C-free diet for 3 weeks. The initial weight of the animal was 190 gm., the final weight 188 gm. The pattern of the epiphyseal plate is irregular and the cartilaginous matrix is loose. "Fibrinoid" material is seen in the distal layers of the cartilage and in the abundant loose fibrous tissue of the metaphysis; the trabeculae are destroyed. $\times 95$.

FIG. 4. Section through the growth zone at the upper end of the tibia of a male guinea-pig kept on a vitamin C-free diet, which received a total of 150 mg. of alpha-estradiol benzoate during this period. Its initial weight of 184 gm. had increased to 286 gm. during the period of observation. The epiphyseal plate is narrowed and intact, the cartilage cells are smaller than in Figure 1, and there is increased hyalinization. The metaphysis contains well formed but slender trabeculae. No "fibrinoid" material and no fibrous tissue are found in the metaphysis. $\times 95$.

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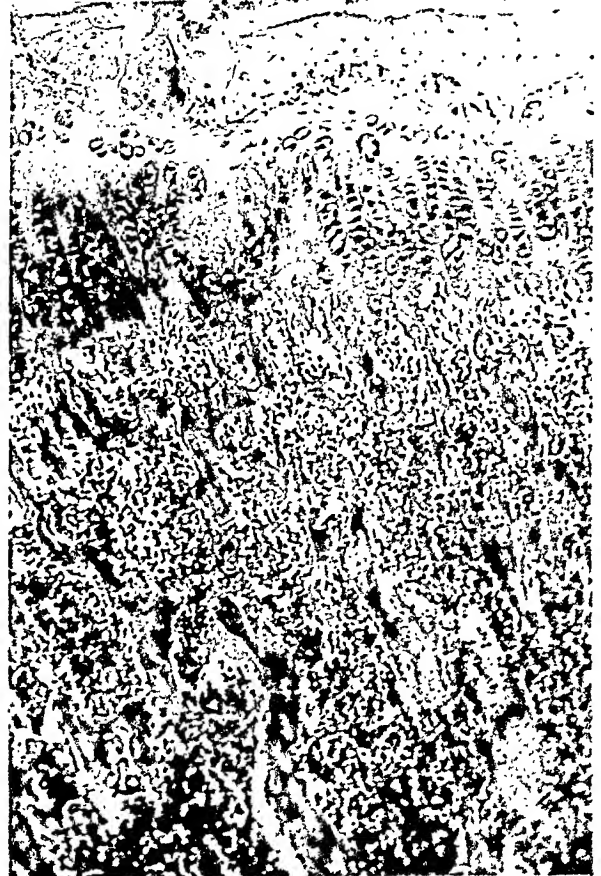


PLATE 168

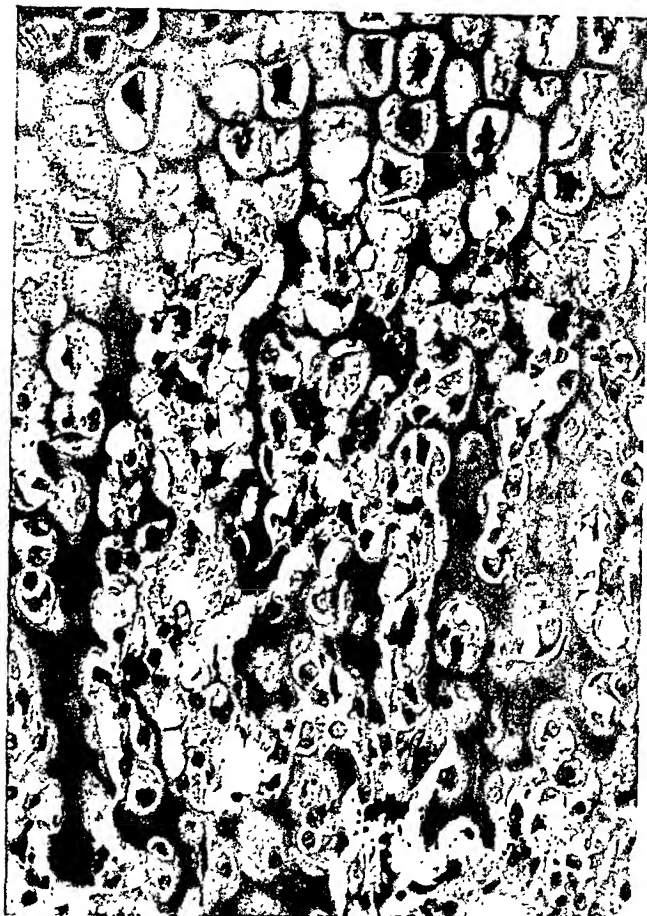
FIG. 5. An area of the metaphysis of the tibia in Figure 1. The hypertrophic cartilage cells are being eroded by engorged capillaries, and many large polyhedral osteoblasts have been laid down along the trabeculae. $\times 285$.

FIG. 6. An area of the metaphysis of the tibia shown in Figure 2. Two hyalinized plugs of degenerated cartilage are seen between the hypertrophic cartilage cells. The latter are smaller and the vascularization is decreased as compared with Figure 5. The trabeculae are long and contain unopened cartilage cells. They are covered by osteoblasts which are smaller and more spindle-shaped than in Figure 5. $\times 285$.

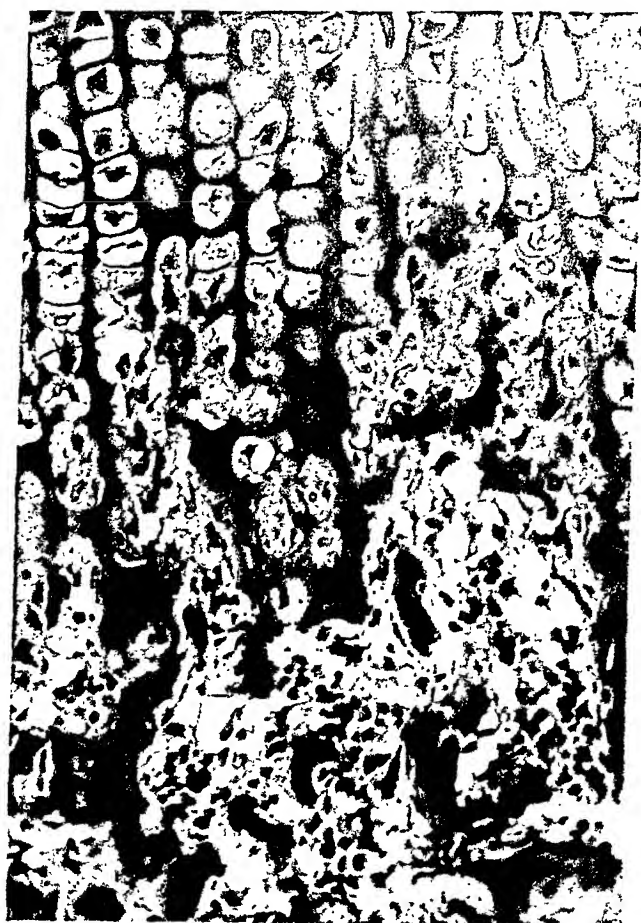
FIG. 7. An area of the metaphysis of the tibia shown in Figure 3. The cartilage cells are distorted and cells of hypertrophic type are absent. The distal part of the cartilage and the metaphysis contain fibrinoid material. There is increased vascularization and enlargement of the capillaries. The primary trabeculae are destroyed. $\times 285$.

FIG. 8. An area of the metaphysis of the tibia shown in Figure 4. The epiphyseal cartilage cells are preserved and better developed than in Figure 7. There is less hyalinization than in Figure 6. Bony spicules are present, but they are shorter and thinner than in Figures 5 or 6. Vascularization is decreased as compared with Figure 7, but increased as compared with Figure 6. Numerous, but small, osteoblasts cover the trabeculae. $\times 285$.

5



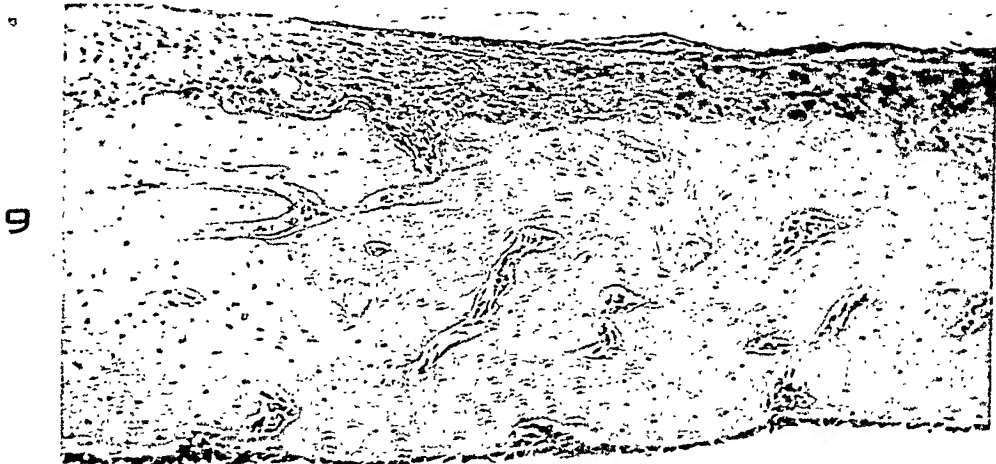
7



8

PLATE 169

- FIG. 9. Section through the cortex of the tibia shown in Figure 1. The endosteum consists of many large osteoblasts; the periosteum is loose and contains osteoblasts and osteoclasts. $\times 95$.
- FIG. 10. Section through the cortex of the tibia shown in Figure 2. Osteoblasts at the endosteum are spindle-shaped and scantier than in Figure 9. The vascularization is decreased, and the osteocytes are small. The outer layer of the shaft is smoother than in Figure 9 and does not contain lacunar grooves with osteoclasts. The periosteum is dense and somewhat less vascular. $\times 95$.
- FIG. 11. Section through the cortex of the tibia shown in Figure 3. The cortex is markedly thinned out, the endosteum consists of spindle cells, the vascular canals are greatly enlarged and contain loose connective tissue poor in cells, and the periosteum is edematous and detached from the cortex. $\times 95$.
- FIG. 12. Section through the cortex of the tibia shown in Figure 4. The cortex is thicker, the vascular canals are less engorged, the connective tissue is more cellular and denser, and the periosteum is less edematous than in Figure 11. $\times 95$.





CYTOLOGIC STUDIES WITH THE PHASE MICROSCOPE
IV. MORPHOLOGIC CHANGES ASSOCIATED WITH THE DEATH OF CELLS
IN VITRO AND IN VIVO *

HANS U. ZOLLINGER, M.D.†

*(From the Department of Pathology of Cornell University Medical College,
and the New York Hospital, New York, N.Y.)*

In this paper changes in dying and dead cells as viewed with the phase microscope (PM) are described. The technic and the material used in these experiments have been described in a preceding paper.¹

Death of an individual means generally "cessation or extinction of life," whereas cell death is interpreted as "complete degeneration or necrosis of cells" (Dorland²). This purely morphologic description of cell death is not satisfactory. Therefore, a method has to be found by which it is possible to test the viability of a cell, thus giving the morphologic statement the necessary biologic background.

OBSERVATIONS

When living cells are suspended in a physiologic medium (saline, buffered glucose Ringer solution (BGR), homologous serum) at 37°C. for from 1½ to 3 hours, the nuclei exhibit a change described as the intermediate stage³: the nuclear membrane becomes thicker and more irregular, the chromatin network is replaced by numerous black, irregular dots of variable size, and the whole nucleoplasm appears somewhat darker (Figs. 1 and 2). In this stage, the cilia of ciliated epithelium gradually move more slowly, and finally stop; but they vibrate again when fresh physiologic saline solution is added to the suspension. Later, more and more nuclei show brilliant nucleoli (Fig. 3), and then the nuclear membrane also becomes brilliant and double-contoured (Fig. 4). The chromatin network of such cells is very plump, some of its condensations (karyosomes) are brilliant, and the nucleoplasm again appears lighter. The protoplasm has shrunk slightly, the mitochondria are medium-sized, and the storage granules⁴ are enlarged (Fig. 5). This can be called the brilliant type of nuclear change.³ These cells, particularly the nucleus and the storage granules, are stained deep red by neutral red in a strength of 1:10,000. Suspensions which have been at 37°C. for 6 hours contain, exclusively, cells with brilliant nuclei.

At room temperature, the cilia of large pieces of tracheal epithelium of frogs and mice still move very fast after 6 hours. Later, they stop

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† Fellow of the Swiss Foundation for Biological-Medical Fellowships.

moving in one cell after the other. At the same time, the first signs of the brilliant type of nucleus appear. After 24 hours, this suspension contains about 70 per cent of cells with brilliant nuclei, and no ciliary movement can be seen after the addition of fresh saline solution.

A cell suspension, after having been in the icebox at 6°C. for 24 hours, shows about 5 per cent of cells with brilliant nuclei, and the storage granules are strikingly small, when compared with those of suspensions stored at higher temperatures. When this suspension is transferred to a water bath at 37°C., the cells show the different stages mentioned above within a much shorter time.

Cells in the mitotic phase contain brilliant, irregular chromosomes after being in the suspension at 37°C. for 30 minutes only. After 1 hour, the chromosomes of the cells in mitosis are no longer visible (see study III³). The mitotic cells with brilliant chromosomes contain relatively large, black protoplasmic granules, and the perichromosomal halo is less distinct than in fresh mitotic cells.

The nuclei of cells situated in the centers of large cell clumps and of tissue fragments turn brilliant long before the single cells and the cells on the edges of clumps.

The cilia of cells with brilliant nuclei never move, and the movement cannot be revived by any means. This was interpreted as indicating that cell death had occurred. In order to obtain a second biologic proof of the death of cells with brilliant nuclei, the following experiment was carried out. Three sterile cell suspensions (C₃H sarcoma, Gardner's lymphosarcoma, and a spontaneous breast tumor of the mouse) were kept in the icebox for 24 hours. Then, after observation with the PM had shown that about 6 per cent of the cells had brilliant nuclei, 0.5 cc. portions of each suspension were injected subcutaneously at different sites into 4 C₃H mice. Another series of 4 C₃H mice were injected with the same amount of similar suspensions, which had been kept at 37°C. for 24 hours; a PM examination before the injection showed that all of the cells of these latter suspensions contained brilliant nuclei.

Examination of the tumor growth by palpation on the seventh day and by autopsy on the tenth day revealed good tumor growth in every site and in every animal of the first series, whereas the tumor cells of the second series, exhibiting brilliant nuclei, did not grow in any animal.

In order to investigate the influence of high temperature on cells, suspensions of Brown-Pearce carcinoma cells in thin-walled test tubes were placed in water baths of different temperatures. Samples of these heated suspensions, the bacteriologic sterility of which was maintained

carefully, were investigated after various intervals of time, and the approximate percentage of cells containing brilliant nuclei was determined. At 37°C. the majority of the cells showed nuclei in the intermediate stage after 2 hours. The mitochondria, and particularly the storage granules, were enlarged. About 15 per cent of the cells contained brilliant nuclei. After 4 hours at 37°C., 54 per cent, and after 5 hours 82 per cent of the cells in the suspension exhibited the brilliant type of nuclear change. One hour later, the suspension contained only a very few cells without the brilliant change of the nucleus. About the same percentage was found when normal rabbit serum was used instead of physiologic saline solution.

A temperature of 50°C. brings about the typical brilliant change of the nuclei in about 32 per cent of the cells after 2 minutes. At this temperature, the mitochondria first enlarge, and later decrease in size. The intermediate stage of nuclear change appears in numerous cells of this suspension. Figure 6 demonstrates such a cell, the nucleus of which turned brilliant 6 minutes after this picture was taken (Fig. 7).

Boiling water immediately produces the brilliant type of nucleus in every cell. The mitochondria and the cellular membrane disappear, and the protoplasm becomes net-like. The cellular membrane also disappears after 10 minutes at 65°C., and after 5 minutes at 75°C. About 10 per cent of the cells exhibit this alteration even after 2 minutes at 75°C., after 5 minutes at 65°C., and after 10 minutes at 56°C.

After 20 minutes the storage granules are slightly enlarged at 37°C. whereas they are small at 45°C. and at 56°C. They disappear after 10 minutes at 65°C., after 5 minutes at 75°C., and immediately in boiling water.

That alcohol, acetone, formalin, and acids also bring about the brilliant type of nucleus has already been described.³ It may be added that the ciliary movement stops in every case as soon as the chemical reaches the cells, and that it cannot be revived by replacement of the chemical by physiologic saline solution. This is true even for the temporary intermediate stage of nuclei in potassium bichromate. The only differences between cells that died spontaneously in suspensions and those killed by formalin, etc., are the thickness of the brilliant nuclear membrane, which is slightly less in the former than in the latter, and the fact that cells that died spontaneously are stained brighter red by neutral red in a strength of 1:10,000 than are the others.

Distilled water produces another type of nuclear change. The nuclei become slightly swollen and the nucleoplasm appears hazy, homogeneous, and gray, whereas the nuclear membranes and the nucleoli remain

distinctly visible. Many hours later, the nuclei may fade, or even disappear (Fig. 8). The cellular membranes and protoplasmic granules of both types disappear much later. In distinction from the brilliant type this is called the hazy type.³ Distilled water does not kill the cells within a short time. The cilia still move very fast, and thus turn the cells over and over, even when the nuclei have been hazy for a few minutes (Fig. 9). Then, the cilia stop vibrating in one cell after another, but not earlier than 12 minutes after the addition of the distilled water (Fig. 10). When distilled water is replaced by physiologic saline solution at this moment, some of the cells renew their ciliary movement, but in many of the cells nothing happens. In distilled water blister formation¹ continues and even starts in cells with hazy nuclei, whereas this phenomenon never occurs in cells with brilliant nuclei. The same is true for the enlargement of the storage granules.⁴

Cilia stop moving when molar NaCl replaces the physiologic medium and the nuclei turn hazy.³ Vibration cannot be revived even if the molar NaCl is replaced by physiologic saline solution just after the nuclei have turned hazy. Cells, the nuclei of which have been brilliant before the addition of molar NaCl, shrink and turn yellow when reached under the coverslip by molar NaCl; the nucleus is no longer visible in such mummy-like cells. There is no similarity between this change and the hazy type, which is produced by the action of molar NaCl on living cells. Formalin (Fig. 11), alcohol, acetone, and acids have the same effect on dead cells.

In order to make absolutely sure about the viability of cells in molar NaCl and in distilled water, a biologic test was used. A bacteriologically sterile suspension of Brown-Pearce carcinoma cells in BGR was prepared and kept at room temperature for 15 minutes. Then, part of it was injected into rabbits (suspension A, control), after it had been noticed with the PM that practically all of the cells were unchanged. From the rest, suspensions B, C, and E were prepared. One and one-half cc. of the original suspension was mixed with 4.4 cc. of distilled water (suspension B); under the PM all of the cells were enlarged, the mitochondria were vesicular, and the nuclei were hazy. Ten minutes later, this suspension was restored to isotonicity by adding an adequate amount of molar NaCl. Under the PM these cells looked absolutely normal. Suspension C (4.5 cc. of BGR mixed with 1.5 cc. of the original suspension) served as a second diluted control. Under the PM all of the cells were found to be as well preserved as in the original suspension A. Suspension D was obtained by pressing the minced tumor pieces through a metal sieve into molar NaCl. All of the nuclei of this suspension were hazy under the PM. An attempt

was made to restore this suspension to isotonicity by means of distilled water (suspension E) but on account of the considerable dilution the suspension contained only a few intact cells, all showing nuclei of the hazy type.

The different suspensions were implanted into 3 adult agouti rabbits. Each animal received intramuscular injections of 1 cc. at six different sites (upper extensors of all four legs, flexors of the thighs): suspension A in three sites; B, C, and D, each in one site. One animal received an injection of suspension E instead of D.

After 9 days, the growth of the tumors was determined by palpation. A tumor of about 3.5 cm. in length was found at each site where suspensions A, B, and C had been injected. At the site of the other injections, no tumor could be palpated. Autopsies confirmed these findings in all animals.

From this experiment it became evident that the Brown-Pearce carcinoma cells were killed by molar NaCl, but that they survived the action of distilled water, although their nuclei had been hazy for at least 15 minutes.

The hazy type of nuclear change also appears in cells of a suspension which remains under the coverslip for several hours without being protected from evaporation (Fig. 12). When fresh physiologic saline solution or BGR is added on the edge of the coverslip, the nucleus turns brighter, the chromatin network as well as the nucleoli reappear, and the cilia start moving again. A somewhat similar alteration is produced by pressure on the coverslip, but in that case the nuclear and cellular membranes are destroyed and the mitochondria disappear, whereas the storage granules remain unchanged (Fig. 13). No signs of life can be seen in such cells, and cilia never move again after the nuclei exhibit this change.

In fresh suspensions of a partly necrotic tumor cellular changes of two further types appear: pyknosis and necrosis. The nuclei of pyknotic cells are very compact, dark, and somewhat granular under the PM. The protoplasmic granules are no longer visible, and the whole cell is more refractive, shiny yellow, and slightly shrunken. Necrotic cells (see Fig. 22 of study II⁴) are very small, irregularly shaped, and shiny yellow, with the exception of rare nuclear fragments which are dark, round particles, 2 to 6 μ in diameter. There is no nucleus to be seen. Every necrotic cell is stained deeply and entirely when neutral red (1:10,000 in physiologic saline solution) is added to the suspension. Such cells do not show any reaction to ammonia and to the other chemicals mentioned above. The transformation of cells containing brilliant nuclei into necrobiotic or necrotic cells does not take place

before the cells have been in the suspension for from 2 to 4 days. The nuclei disappear by karyorrhexis or karyolysis. In very fresh suspensions of partly necrotic tumors, intermediate stages between brilliant nuclei and pyknotic or necrotic cells occasionally are seen.

DISCUSSION

Cowdry⁵ defined death as "the disorganization of living matter, which makes permanently impossible all vital phenomena." One of these vital phenomena, which can be observed under the PM, is the movement of the cilia of the ciliated epithelium of the frog's pharynx. This motion is an indication of the viability of a cell, whereas the lack of ciliary movement suggests the death of a cell, provided that the restoration of the suspension medium to normal conditions does not engender further movement of the cilia. A second and more trustworthy indication of viability is the multiplication of malignant tumor cells after their injection into susceptible hosts. As a matter of fact, the experiments mentioned above show that the same adverse surroundings under which cells suffer loss of the vegetative function of ciliary movement also cause loss of the ability of malignant tumor cells to grow in susceptible hosts.

Furthermore, these studies with the PM demonstrate that various morphologic alterations of the nuclei develop simultaneously with the loss of the viability of cells. Cells which have been in a physiologic medium for several hours first show the intermediate stage of nuclear change,³ and then turn brilliant.³ This increase of the nuclear refractivity and the appearance of a marked nuclear membrane are considered by Lewis and McCoy⁶ as typical signs of cell death. The biologic test mentioned above confirms this assumption. The viability of cells showing nuclei in the intermediate stage could not be tested, since suspensions never contain this cell type exclusively.

It was shown⁴ that the storage granules in dying cells probably carry waste products of the cells. The enlargement of the storage granules is distinctly accelerated at relatively high temperatures, considerably delayed at low temperatures, and even totally suppressed in the icebox. This suspended or delayed accumulation of waste products at low temperature (vitrification) may be one explanation of the prolonged survival time of the cells, and it is known that cells can be revived after having been in a state of total vitrification for weeks and even months.

The beginning of the brilliant type of nuclear change indicates that cell death has already occurred. Baker⁷ and Bradley⁸ showed that the intracellular pH becomes acidic immediately after death, and the

experiments with acids³ demonstrated the same qualitative effect of acids on the nuclear elements as observed in spontaneous death. The slight qualitative difference between cells which died spontaneously and those which were artificially killed, which consists in a smaller width of the nuclear membrane in the former, is presumably a consequence of the higher precipitative potency of these chemicals.

It has been known for a long time that the nuclei of dead cells in suspensions are stained diffusely by neutral red, etc., whereas they are not stained during life. Schrek⁹ stated that formalin-killed cells do not take eosin (1:2,000), whereas cells which die spontaneously are stained red. In my experiments when eosin was used one could observe a slight delay in the staining of formalin-killed cells and a paler color. Dead cells of both types were well stained by neutral red in a 1:10,000 solution.

From the observations on the death of cells by heat it is clear that the percentage of dead cells depends not only on the temperature, but also on the time during which the hot solution acts upon the cells. Strong heat, even 75°C., apparently does not kill all of the cells of one particular type in a suspension at the same moment. It is conceivable that this is due to differences in the sensitivity of the single cells, depending on their age and their stage of degeneration previous to the heat experiment.

The hazy type of nuclear change, which is produced by distilled water, molar NaCl, ammonia, alkali, and mechanical destruction of the cells, is not a reliable sign of death since cells which show the hazy type of nuclei caused by distilled water can be revived. On the other hand, when the hazy change of nuclei is caused by any other of the above-mentioned agents, these cells cannot be revived, and should be considered dead. It is also of interest that the appearance of the hazy type of nucleus proves that the cells have been living before the agent acted upon the cells, because previously dead cells are converted into yellow mummies when exposed to these agents, and do not show hazy nuclei (Fig. 11).

Contrary to the opinion of Lewis,¹⁰ blisters are not a sign of death. In fact, the initial formation, or a further growth of blisters in dead cells, has not been seen, and the majority of dead cells do not contain blisters. Cells which have already formed blisters may retain them after death, but the formation or the further enlargement of already existing blisters is a reliable sign of life.¹

The movement of the cilia is another very reliable indication of the viability of cells. When the cells die, it stops and the nuclei turn brilliant simultaneously; the movement can never be revived in such cells.

In cells containing hazy nuclei produced by the action of distilled water or molar saline solution, the ciliary movement corresponds with the outcome of the biologic test. In distilled water, the cilia move for a time, or if this movement has stopped it usually can be revived by the action of normal saline solution. Accordingly, malignant tumor cells treated with distilled water for a limited time do not lose their ability to grow in susceptible hosts. In molar saline solution, on the other hand, the ciliary movement stops irreversibly, and the cells do not grow in hosts.

With respect to the protoplasm of dying cells, the descriptions offered by Lewis¹⁰ and Lewis and Lewis¹¹ are somewhat different from the observations reported above. These authors mentioned that the mitochondria, as well as the "degeneration granules," and the vacuoles disappear, and that their disintegration is followed by granules of a new type, called "death" or "d-granules" by Lewis.¹⁰ However, the appearance of granules of a new type was never observed in the experiments mentioned above, and the mitochondria did not disappear until the nucleus broke down. The storage granules were still well preserved, but very much enlarged, even when the cells disintegrated in physiologic saline solution at 37°C. after 1 day. The "death granules," therefore, are likely to be identical to the enlarged storage granules. On the other hand, one has to take into consideration the fact that the observations of these authors are based on a material different from that used in my experiments.

The degenerative protoplasmic alterations, as seen in cells in old suspension, and the intermediate stage of nuclear change in such cells, remind one very much of cloudy swelling (see, for instance, the description given by Karsner¹²). Water-intake is considered to be the first step leading to cloudy swelling. In cell suspensions it manifests itself by blister formation,¹ but in tissues the entire cell becomes swollen. Karsner suggested that acidification of the protoplasm could be the reason for these changes. This opinion corresponds to the explanation of the final stages of cell death given above. The fact that cells in the mitotic phase die much sooner in suspensions than do resting cells is a further proof of the increased sensitivity of cells during mitosis.

The mummy-like cells, produced by the action of various chemicals upon already dead cells, are generally called necrotic if viewed in microscopic sections, and, as a matter of fact, cells of necrotic tumor areas show a similar structure to these mummies when viewed with the PM. The small structural differences between genuine necrotic cells in tissues (*in vivo*) and mummy-like cells which develop under the influ-

ence of fixatives from fresh dead cells (*in vitro*) are likely to be based upon differences in the manner of action of the protoplasmic autolytic enzymes and of the chemicals added to the suspension, both of them altering the already dead cells. According to Baker,⁷ only the cleavage of the nucleoproteins into amino acids by enzymes brings about the typical structure of necrotic cells. Therefore, the disappearance of the nuclei in stained microscopic sections of necrotic areas does not signify more than the fact that the resorption of dead cells has already started. Recent cell death as such cannot be recognized in microscopic sections, since all of the cells are killed anyway during the process of fixation. Small wonder that by means of the ordinary microscope one cannot recognize any alterations in sections of very fresh infarcts of the myocardium, etc. It can be expected that the PM will be of valuable practical help for future studies in this field, because it discloses even the earliest changes indicating death.

What is the significance of pyknosis and necrobiosis in general? It already has been stated that the development of neither pyknosis nor necrosis can be observed in short-term experiments *in vitro*, provided that no chemicals are added; the further changes of dead cells in suspensions consist of a decrease in nuclear size, and later of karyorrhexis, a very slow disintegration of the storage granules (Fig. 14), and disappearance of the mitochondria. In fresh cell suspensions, it is possible to find the various stages of nuclear disintegration, which has taken place *in vivo*. The earliest stage is the brilliant type; then follow pyknosis, karyorrhexis, and karyolysis (necrosis). Accordingly, pyknosis—one of the various signs of necrobiosis—as well as necrosis itself, are stages in a consecutive series of secondary changes in dead cells.

SUMMARY

The phase microscope makes possible a method by which the cytology of dying and dead cells can be studied without interference by the effects of fixation and staining.

The most conspicuous alterations indicating cell death occur in the nucleus, as described in a preceding paper.³ One can distinguish between a *brilliant* and a *hazy* type. A third alteration, the temporary *intermediate stage*, is produced by 3 per cent potassium bichromate; it can be seen also in old cell suspensions. The brilliant type, caused by over-age of the suspension, heat, fixatives, or in other ways, is a reliable sign of death. The viability of cells exhibiting the intermediate stage of nuclear change could not be tested. The hazy type following the action of distilled water does not, as such, prove cell death, because such cells in distilled water can be revived. With molar NaCl,

ammonia, alkali, and mechanical crushing of cells, hazy nuclei indicate cell death.

In addition to the nuclear structure, the ciliary movement is an excellent morphologic indicator for the viability of cells; the movement stops irreversibly and simultaneously with the appearance of nuclear signs of death. The same is true for the new formation and the further growth of blisters. Cells of transplantable tumors do not grow in susceptible hosts if they show the above-mentioned morphologic signs of death.

Mitotic cells die much sooner in suspensions, and they are generally more sensitive to artificial changes of the surrounding milieu than are cells in the resting phase.

Necrotic and necrobiotic cells are dead cells that show alterations caused by the action of autolytic intracellular enzymes in addition to the nuclear and protoplasmic changes mentioned above. Necrosis and necrobiosis occur only after 2 to 4 days *in vitro* under the experimental conditions herein described.

It is conceivable that cloudy swelling of cells *in vivo* is identical to the first stage of degeneration observed in cells in suspensions.

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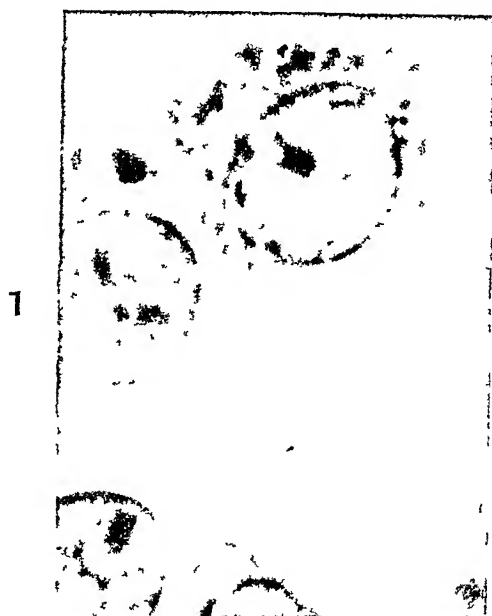
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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 170

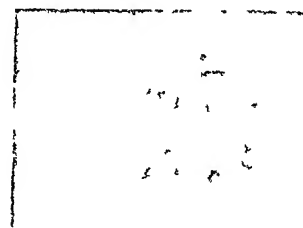
- FIG. 1. Brown-Pearce carcinoma cells which have been at 37°C. for 90 minutes. There are large blisters in both cells on the left, and some vacuoles in the right upper cell. The chromatin network begins to appear more granular in the two cells on the left, when compared with the upper cell. PM. $\times 1400$.
- FIG. 2. Brown-Pearce carcinoma cells, which have been in a suspension at 37°C. for 2½ hours. The two cells in the left upper quadrant show the intermediate stage of nucleus; in the nucleus below, this change is just beginning. PM. $\times 1400$.
- FIG. 3. Dying Brown-Pearce carcinoma cells, which have been at 37°C. for 4 hours. The chromatin appears more like spots than in the form of a network, the nuclear membranes are more irregular and thicker than in normal cells, and the nucleolus of the lower left cell is already brilliant. PM. $\times 1400$.
- FIG. 4. C₃H sarcoma cells, later stage of death (4½ hours). The two cells on the right are dead, showing an irregular, double-contoured, brilliant membrane and brilliant nucleoli. The cell on the left shows a slight wrinkling of the nuclear membrane, and enlarged chromatin condensations. Of note are a large blister and greatly enlarged storage granules of the upper of the two dead cells. PM. $\times 1400$.
- FIG. 5. Brown-Pearce carcinoma cell, last phase of death (6 hours), showing shrinkage of the nucleus. A previously existing blister has not disappeared. PM. $\times 1400$.
- FIG. 6. Brown-Pearce carcinoma cell, which has been at 50°C. for 2 minutes. The nucleus is slightly shrunken, the nuclear membrane is irregular, the chromatin network is dense, and the nucleoplasm has become cloudy. PM. $\times 1400$.
- FIG. 7. The same cell as shown in Figure 6, 6 minutes later. The nuclear changes are still more distinct, the nuclear membrane and the nucleolus are brilliant, and the storage granules are enlarged. PM. $\times 1400$.
- FIG. 8. Late phase of the hazy changes. Ghost cells, with but little recognizable cellular structure, are seen. PM. $\times 1400$.



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4



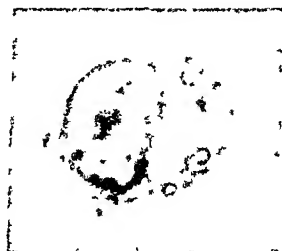
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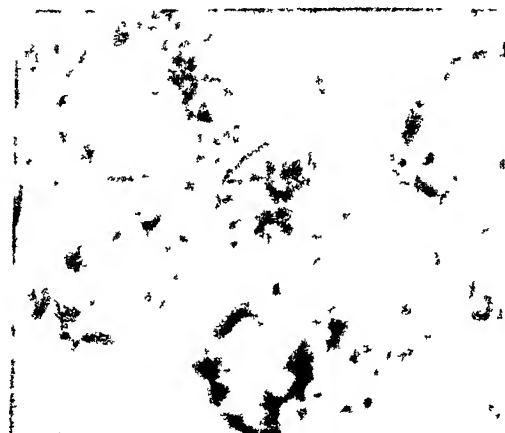
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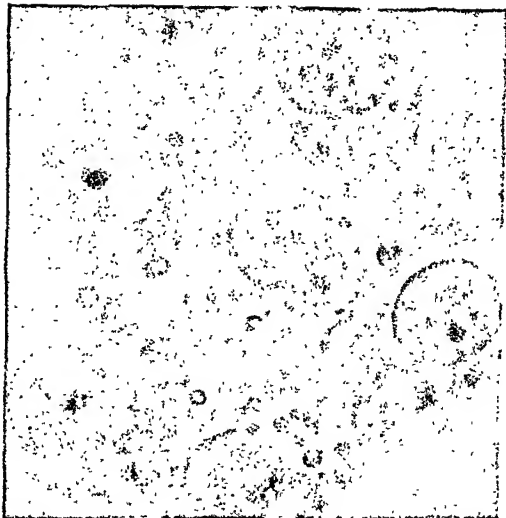
PLATE 171

- FIG. 9. Ciliated epithelial cell of the frog pharynx after having been in distilled water for 3 minutes. The cilia move so fast that the cell constantly turns around and, therefore, appears indistinct. The nucleus is already hazy and enlarged; the nucleolus of the cell on the right is visible. PM. $\times 1400$.
- FIG. 10. The same cell as shown in Figure 9, 8 minutes later. The cilia no longer move; a large blister has appeared; vacuoles have formed; and the nuclei are hazy. PM. $\times 1400$.
- FIG. 11. The effect of 10 per cent formalin upon living and dead cells. The nucleus of the uppermost cell was already brilliant before formalin was substituted for the physiologic saline solution; the nuclei of the other cells are unchanged. The formalin has just reached the cells: the nuclei of cells previously living show the intermediate stage, whereas the dead cell is shrunken, and has become more refractile. PM. $\times 1400$.
- FIG. 12. Frog kidney cells after the same sample of the suspension was observed under the coverslip for 45 minutes. The cells are beginning to change their appearance because of evaporation of the suspension fluid. The nucleoplasm of the two cells on the left has become hazy and almost homogenous, whereas the nucleoli are unchanged. The storage granules and the mitochondria are enlarged. PM. $\times 1400$.
- FIG. 13. Brown-Pearce carcinoma cells adjacent to a crushed area caused by local pressure on the coverslip. The nuclei are hazy, but, with the exception of the cell on the left, they are distinctly outlined; the nucleoli have disappeared. The granules are decreased in number, and the protoplasm contains numerous small vacuoles. PM. $\times 1400$.
- FIG. 14. Brown-Pearce carcinoma cell, which was observed for 36 hours after spontaneous death. The nucleus is darker and smaller than in recently dead cells. The majority of the storage granules have disappeared and the mitochondria are no longer recognizable. PM. $\times 1400$.

9



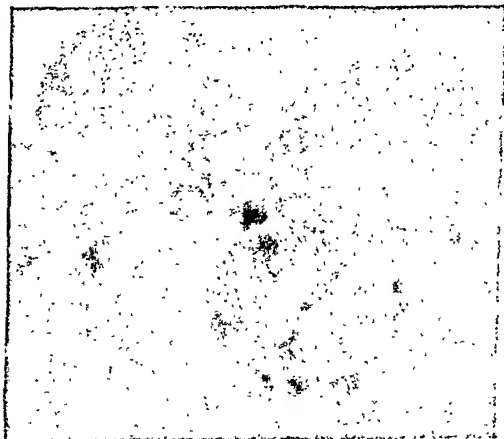
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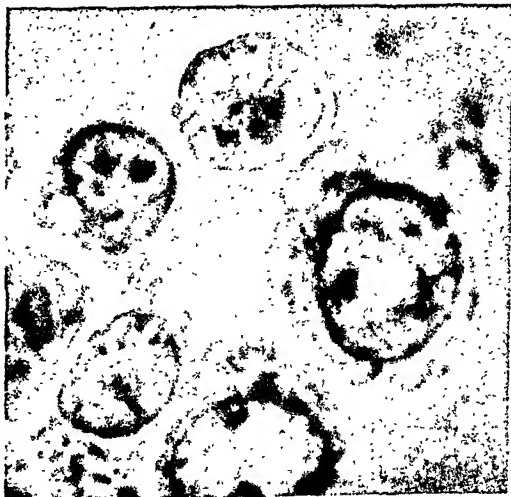
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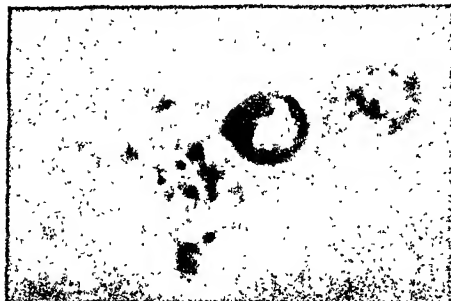
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11



14



Zollinger

Phase Microscopy, Cellular Death

CYTOLOGIC CHANGES IN THYMIC GLANDS EXPOSED IN VIVO TO X-RAYS *

ROBERT SCHREK, M.D.

(From the Tumor Research Unit, Veterans Administration, Hines, Ill.)

In previous work,¹⁻⁴ cellular suspensions derived from the thymic glands of rabbits were treated with x-rays and were studied by the method of unstained cell counts, by dark-field microscopy, and by histologic sections. It was found that x-rays killed the thymic cells after a latent period of 3 hours or more. During the latent period, many of the viable, irradiated cells developed single or multiple structures which were visible on dark-field illumination and which were termed primary vacuoles. In histologic sections of the suspension, the primary vacuoles were seen to be intranuclear. Nearly all of the irradiated cells were dead after 24 hours of incubation at 37°C. Dark-field examination showed that the dead cells were small and round and had single, fairly large, dark, partly encapsulated structures which were termed secondary vacuoles. In histologic sections the secondary vacuoles were seen to correspond to the pyknotic nuclei. Further study showed that in nonirradiated suspensions incubated at 37°C., primary and secondary vacuoles also developed but at a slower rate than in irradiated suspensions. It was concluded that x-rays did not initiate any new degenerative process but accelerated the normal ageing, degeneration, and death of the lymphocyte.

These observations on the action of x-rays were made on thymic cells which were irradiated *in vitro* and were incubated aerobically at 37°C. The present study considers the question whether similar phenomena occur in thymic tissue irradiated *in vivo*.

METHODS

Anesthetized rats and rabbits were treated with 1000 r. units of x-ray irradiation over the thymic glands and the animals were sacrificed 1 to 6 hours later. One lobe of the gland was fixed in Bouin's solution and other fixatives. Paraffin sections of the tissue were stained with hematoxylin and eosin and with Feulgen's stain.

The other lobe of the irradiated gland was chopped up with scissors and filtered through a fine metal screen. To a small sample of the resulting suspension was added eosin in Tyrode's solution and a count was made of the number of stained (or dead) cells, unstained (or

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viable) cells, and red blood cells. By this method of unstained cell counts, it was possible to estimate the percentage of viable cells in the suspension. The suspension was examined also by dark-field illumination to determine the presence of primary and secondary vacuoles. Further details of the methods used have been presented in previous papers.¹⁻⁴

IRRADIATED THYMIC GLANDS

An unstained cell count of the suspension of a thymus, 3 hours after irradiation, showed that 85 per cent of the cells were viable (Fig. 1). In a control suspension of a normal thymus, 86 per cent of the cells were viable. Evidently, the technical procedures killed approximately 15 per cent of the cells in the two suspensions. Apparently the lymphocytes were not killed by the x-rays 3 hours after irradiation.

On dark-field examination of the irradiated cells, 35 per cent of the lymphocytes had primary vacuoles which appeared as single or multiple, dark, round structures usually surrounded by a vacuolar wall (Fig. 2). Some of the cells with multiple vacuoles were irregular. The vacuolated cells were similar in all respects to those seen in suspensions irradiated and incubated *in vitro*. In contrast, the suspensions from normal thymic glands had only an occasional vacuolated cell. The normal cells were round and were filled with fine intranuclear granules and had a few larger, brighter mitochondria (Fig. 3).

In the sections stained by Feulgen's method (Fig. 4), many nuclei had a round, central vacuole which either was unstained by Feulgen's method or took a light homogeneous stain. An occasional cell had two such clear areas. Surrounding the vacuole was a basophilic mass in the shape of a ring, horseshoe, or crescent. This mass usually stained deeply and uniformly, but in some cells it was composed of numerous fine chromatin granules.

Sections stained with hematoxylin and by Giemsa's method also showed cells with vacuolated nuclei. There appeared to be, however, fewer vacuolated nuclei in sections stained with hematoxylin than in those prepared by Feulgen's method.

On correlating the appearance of the living and the fixed cells, it would appear that the vacuoles observed on dark-field study corresponded with the unstained central areas in the sections. The ring or horseshoe-shaped, Feulgen-positive mass surrounding the vacuoles in the fixed sections probably corresponded with the bright, thick vacuolar wall observed under dark-field examination.

Thymic tissue removed 5 hours after irradiation showed, in histologic sections, few normal nuclei in the cortex. Most of the cells had pyknotic nuclei and a few had intranuclear vacuoles. In addition there were also many fragmented nuclei which stained deeply.

A suspension derived from this 5-hour thymus was prepared simultaneously. Examination of the eosin-stained cellular suspension showed numerous small, protoplasmic masses which varied in size down to $1\ \mu$ in diameter. The protoplasmic masses usually were unstained. They evidently corresponded to the fragmented nuclei seen in histologic sections. In spite of the failure of the small protoplasmic masses to stain with eosin, one had to assume that they were not viable. The failure of the protoplasmic masses to stain and the difficulty in differentiating a complete cell from a cell fragment made it difficult to obtain a satisfactory unstained cell count.

Dark-field examination of the suspension showed many cells with a single, large, secondary vacuole and a small crescent of fine and coarse granules. A thick, bright wall separated the vacuole from the surrounding media but there was no wall between the vacuole and the cytoplasmic granules. The fragmented nuclei seen in the sections and the unstained small protoplasmic masses observed in the hemacytometer were found, on dark-field illumination, to have thin but well illuminated walls (Fig. 5). Most of these structures were dark, but some of them had a few small granules which did not show brownian movement.

NORMAL THYMIC GLANDS

According to the unstained cell counts, suspensions of untreated thymic tissue contained cells of which 14 per cent were dead and stained readily with eosin. It is believed that most of the dead cells were killed in the course of preparing the suspension. On dark-field examination a rare cell had a primary or secondary vacuole. In histologic sections an occasional cell with an intranuclear vacuole was found (Fig. 6). Evidently the degeneration of cortical cells by means of intranuclear vacuoles occurred *in vivo* in normal, nonirradiated thymic glands.

DISCUSSION

Intranuclear Vacuoles

These studies by dark-field illumination showed that the first histologic reaction of lymphocytes to irradiation was the formation of one or a few small primary vacuoles within the nucleus. The formation and growth of the vacuoles were associated with an observed increase in the size of the cell.

Vacuolization and increase in the size of nuclei following irradiation probably have been observed by many pathologists in routine surgical and post-mortem tissue sections. As early as 1907, Warthin⁵ described vacuolation of the nuclei of renal epithelium following irradiation. A good description of the phenomenon was given by Eckert and Cooper.⁶ They observed that normal and malignant epithelial cells

of the uterine cervix underwent, after irradiation, three changes in the nuclei, namely, pyknosis, enlargement, and vacuolization. They found that the least common aberration in normal cells was vacuolization. The vacuoles varied in size up to the point of occupying the entire nucleus. They also varied in position and were either central or eccentric. Those located eccentrically were found usually in elongated nuclei and frequently were large enough to distend and distort one end of the nucleus. Occasionally, two or three vacuoles appeared in a single nucleus. In 3 cases of carcinoma of the cervix, the most notable change following irradiation was the presence of many vacuolated nuclei. The neoplasms of these cases received relatively small amounts of roentgen irradiation and all showed little effect from the therapy other than the increased vacuolation. The Feulgen stain was found to reveal more nuclear damage than the hematoxylin and eosin stain. Eckert and Cooper stated they could not explain the vacuolation of the nuclei, but suggested that the vacuoles were forms of localized degeneration of the nuclei.

Intranuclear vacuolization following irradiation has not, however, been described by some investigators. Akaiwa and Takeshima,⁷ for example, irradiated the popliteal lymph nodes of rabbits. They found that during the first 12 hours the lymph node became enlarged and many cells showed nuclear disintegration. Intranuclear vacuoles were not observed although one would expect them, according to the present studies. The failure to observe post-irradiation, intranuclear vacuoles may be attributed to several factors. In the first place, these vacuoles are transient and the time of examination should be suitable for their demonstration. Secondly, the usual methods of fixation cause more or less shrinkage of cells and are not satisfactory for the preservation of intranuclear vacuoles. Finally, the predominating lesion in the irradiated tissue is the end-result of pyknosis and fragmentation, rather than the intermediate stage of vacuolization. As a result of these factors, investigators have overlooked or failed to describe intranuclear vacuolation. It is only when dark-field methods are employed that one realizes the high incidence of these vacuoles in irradiated cells.

Intranuclear Inclusions

To some extent, intranuclear vacuoles resemble intranuclear inclusions. A satisfactory definition differentiating between vacuoles and inclusions cannot at present be given, since the nature of these two structures is not known.

Intranuclear vacuoles have been observed in normal tissue and in tumors, according to the work of Roskin,⁸ Osgood,⁹ Apitz,¹⁰ and others.

Similarly, intranuclear inclusions have been described in normal and malignant tissue. Bland and Russell¹¹ found intranuclear inclusions in tissue cultures of human meningiomas. They were visible in the living nucleus and had the appearance of vacuoles which closely resembled cytoplasmic vacuoles. They were optically empty by transmitted light but appeared granular by ultraviolet light. They could be seen by means of the dark-ground method. These authors, however, did not describe the appearance of the inclusions under dark-field illumination. In cultures stained with hematoxylin, the inclusions appeared as vacuoles limited by a membrane. Their internal structure was slight and consisted of a fine granulation or a spider's web of thin threads which stained pink with eosin. It would seem from this description that the intranuclear structures might be called vacuoles just as aptly, or perhaps even more suitably, than inclusions.

Acidophilic intranuclear inclusion bodies in human gliomas were reported by Russell.¹² Similar structures were found by Wolf and Orton¹³ in glioblastomas and in meningeal and perineural fibrosarcomas. Ludford¹⁴ observed inclusion bodies with and without melanin in tissue cultures of human melanomas.

Fischmann and Russell¹⁵ described inclusions in tissue cultures of human leptomeningioma, of rat and chick embryos, and of fibroblasts from the lungs of human and rat fetuses. Attempts to influence the development of the inclusions were unsuccessful. These authors also observed deep indentations or bays within the nuclear membrane so that the nucleus appeared as an incomplete ring. Similar structures were observed in the present study and described as horseshoe-shaped masses of chromatin material. Fischmann and Russell considered the possibility that the incomplete-ring nucleus closed and nipped off a cytoplasmic mass which became the intranuclear inclusion. In this study, it seemed that the ring nucleus ruptured at one point and ultimately discharged the contents of the vacuole into the cytoplasm. The present findings indicated that the ring nucleus preceded the horseshoe-shaped nucleus, and not the reverse as suggested by Fischmann and Russell.

Lucké¹⁶ described prominent intranuclear inclusions in a tumor of the kidney of the frog, *Rana pipiens*. Covell¹⁷ observed inclusions in tissues of apparently healthy monkeys. Both believed that the inclusions indicated the presence of a virus.

In view of the present studies, one might consider the possibility that the inclusions occurring spontaneously in normal and malignant cells were, like intranuclear vacuoles, signs of spontaneous degenerative changes in the cells.

Intranuclear inclusions or vacuoles have been produced by the use of various injurious agents, such as distilled water intravenously and salyrgan intramuscularly (Lee¹⁸), bismuth preparations (Pappenheimer and Maechling¹⁹), lead (Blackman²⁰), aluminum and ferric compounds (Olitsky and Harford²¹), and burns (Belt²²). Some of the structures produced by these agents had the typical features of intranuclear inclusions, namely, deep acidophilic staining, halo formation, and margination of chromatin. The inclusion bodies produced by some of these agents may be related to the inclusions that arise spontaneously in cells and to the vacuoles that are induced by x-rays.

The type form of intranuclear inclusions is that which occurs in virus diseases. The chemical attributes and the pathogenesis of these structures are not known. Cowdry and Kitchen²³ observed that in livers affected by yellow fever, many cells had empty and vesiculated nuclei. The empty nuclei were greatly enlarged, with clear, structureless centers, and appeared to be somewhat similar to those induced by hypotonic solutions in a previous study.³ The vesiculated nuclei were not enlarged and had one or more vesicles of small and moderate size. The published descriptions of the vesiculated nuclei are similar to the vacuolated nuclei observed in irradiated thymic tissues in the present study. Intranuclear inclusions, empty nuclei, and vesiculated nuclei occurred together in the livers of patients with yellow fever. The coexistence of these three nuclear changes suggests a possible relationship in the pathogenesis of virus-induced inclusions and the vacuoles produced by spontaneous and induced degenerative changes.

It would appear that early workers sometimes failed to differentiate between intranuclear vacuoles and inclusions. More recently, Cowdry²³ and others have provided criteria for recognizing inclusions. The question remains whether there is any relationship between the two forms of nuclear degeneration. A study of inclusions and vacuoles by means of dark-field illumination may aid in demonstrating differences or similarities.

Degenerative Changes in Irradiated Lymphocytes

It is well known that the end-results of irradiation of lymphoid tissue are pyknosis, karyorrhexis, and karyolysis. The present work showed that an early or intermediate stage is the intranuclear vacuole. The course of events in the degeneration of a normal nucleus may be surmised from the dark-field observations and from examination of sections of an irradiated thymic gland. The hypothecated stages in the changes of the irradiated lymphocytic nucleus are shown in Figure 7. This figure was drawn from sections of a thymic gland of a rat

excised 3 hours after irradiation of 1000 r. Apparently the first manifestation of a change in the irradiated cell (Fig. 7, B) was a decrease in the staining of the central part of the nucleus and a shifting of the chromatin granules towards the periphery. At this stage, the nucleus had a small, lightly stained central zone and a thick ring of discrete chromatin granules. Later (D), there was an increase in the size of the central area and a decrease in the thickness of the ring, which now stained deeply and uniformly. The formation and growth of the intranuclear vacuole were probably associated with an enlargement of the nucleus. The continued increase in the size of the vacuole finally ruptured the ring of basophilic material, with the formation of a horseshoe-shaped mass (E and F). The outline of the nucleus could still be made out because of the presence of a thin, lightly stained nuclear wall. The contraction of the horseshoe-shaped mass produced first a crescent (G) and finally the small spherical pyknotic nucleus (H).

SUMMARY

Intranuclear, acidophilic vacuoles were observed in many cells of thymic tissue of the rat and rabbit, 3 hours after irradiation with 1000 r. of x-rays. On dark-field examination of the suspension of the irradiated thymic tissue, many of the cells had single or multiple primary vacuoles with or without vacuolar walls. Thymic tissue, 5 hours after irradiation, had, on histologic examination, many pyknotic and fragmented nuclei. Suspensions of this tissue had many cells with secondary vacuoles and many fragments of cells. Nonirradiated thymus also had a few cells with intranuclear vacuoles and pyknotic nuclei. The spontaneous and x-ray-induced degeneration of lymphocytes was associated with the formation of intranuclear vacuoles, pyknosis, and fragmentation. Primary intranuclear vacuoles are seen in a higher percentage of cells by dark-field methods than by routine histologic technic.

ADDENDUM

Two recent publications have described nuclear structures which may be similar to the intranuclear vacuoles reported in this paper. Rachmilewitz and co-workers²⁴ studied the effect of irradiation on bone marrow in tissue culture. The first sign of nuclear damage in erythroblasts and normoblasts appeared to be the accumulation of the chromatin at the periphery of the nucleus with a formation of a clear central zone. The nucleus was thus transformed into a large, almost colorless, sharply outlined vesicle. Dustin²⁵ made a detailed cytologic study of cells treated with various mitotic poisons. In intestinal epithelial cells of animals treated with hydroquinone, the degeneration of the nuclei resembled pyknosis in lymphoid tissue. The thymonucleic acid was found to accumulate on the nuclear membrane with the formation of a sphere composed of acidophilic protein and ribonucleoprotein. It is not definite from these publications whether the degenerative process with formation of a vesicle or sphere begins as a focal or diffuse process. Dark-field

studies suggest that the earliest change is a focal involvement with the formation of a small vacuole. This rapidly enlarges and involves the entire nucleus.

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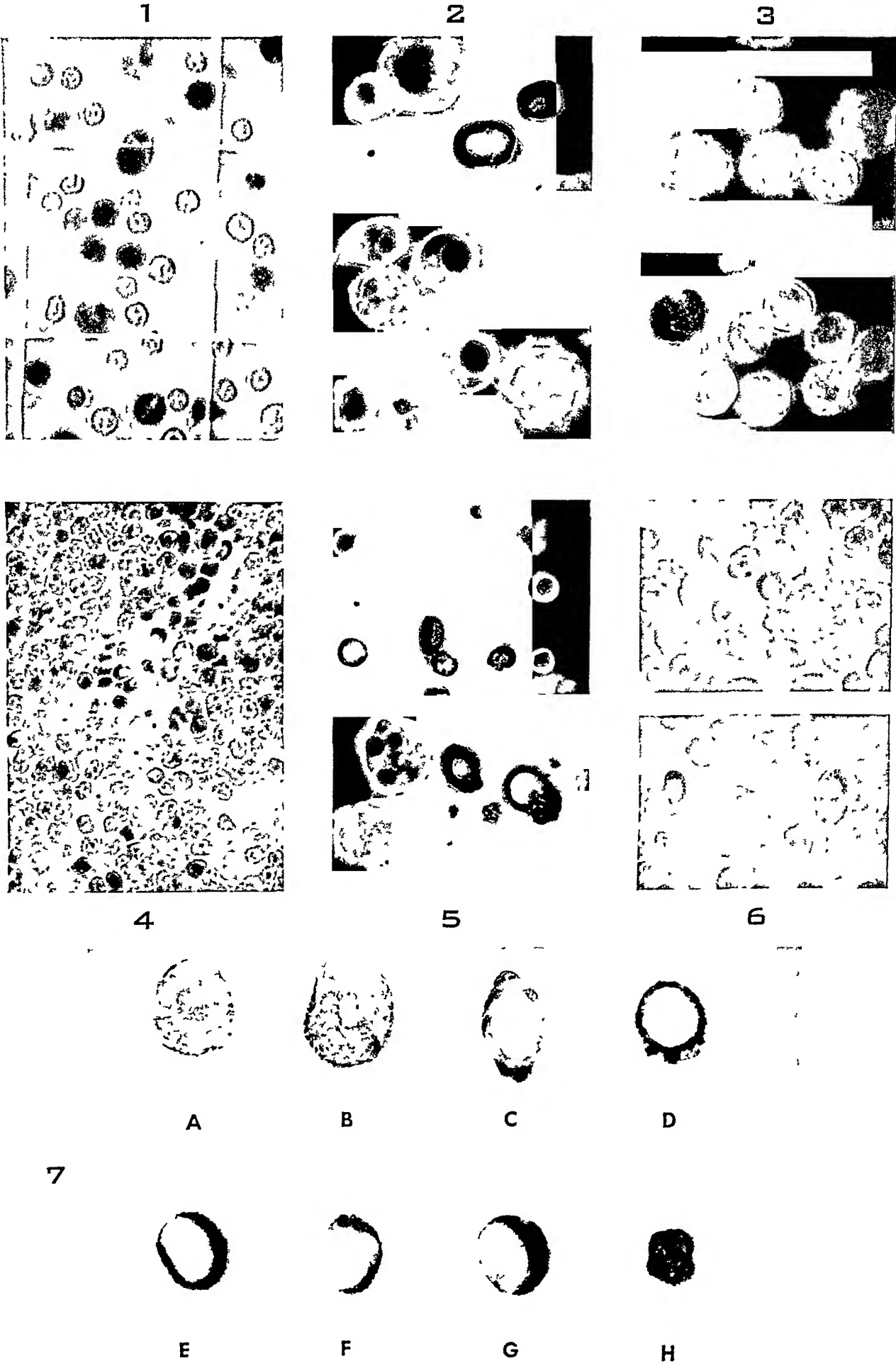
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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 172

- FIG. 1. A mixture of an eosin solution and a suspension of a rat thymus removed 3 hours after irradiation (1000 r.) *in vivo*. The mixture is in a hemacytometer. Most of the cells are unstained, have fine granules, and are presumably viable. The stained cells are larger than the unstained, take a uniformly deep stain, and are presumably dead. $\times 550$.
- FIG. 2. Dark-field view of cells in a suspension of a rat thymus removed 3 hours after irradiation with 1000 r. *in vivo*. One cell in the upper photomicrograph and three cells in the lower have primary vacuoles which appear as large, round structures with or without a thin vacuolar wall. $\times 1250$.
- FIG. 3. Dark-field view of cells in a suspension of a normal rat thymus. The cells are round and filled with fine intranuclear granules. In one cell in the lower picture, the nuclear wall can be seen clearly. $\times 1250$.
- FIG. 4. Histologic section of a rabbit thymus excised 3 hours after irradiation *in vivo*. A few nuclei have small or large, central, unstained vacuoles surrounded by a ring or horseshoe-shaped mass of dense chromatin material. The section is stained by Feulgen's method. $\times 710$.
- FIG. 5. Dark-field view of cells in a suspension of a rat thymus removed 5 hours after irradiation (1000 r.) *in vivo*. The cell on the right side of the lower field has a large, eccentric, secondary vacuole and a mass of bright granules. The upper field shows a group of small, round masses with prominent walls and dark centers. These masses presumably represent fragments of nuclei which developed *in vivo* and are the result of irradiation. One cell in the lower area has three small, primary vacuoles. $\times 1250$.
- FIG. 6. Sections of the thymus from a normal rabbit. One cell in the upper picture and two cells in the lower picture have large, unstained vacuoles surrounded by horseshoe-shaped masses of dense chromatin material. $\times 1250$.
- FIG. 7. Drawings of nuclei found in a section of a rat thymus removed 3 hours after irradiation. The nuclei are arranged to represent the assumed course of events in the transformation of the normal nucleus to a pyknotic one. (A) Normal nucleus with fine granules. (B) Nucleus with small, central vacuole surrounded by a thick ring of finely granular, nuclear material. (C) and (D) Nuclei with large vacuoles surrounded by a thin ring of dense, structureless, basophilic material. (E) Rupture of basophilic ring with the formation of a large intranuclear vacuole surrounded by a horseshoe-shaped mass of basophilic material. The nuclear wall persists between the legs of the horseshoe-shaped mass. (F) and (G) Contraction of the chromatin material with the formation of a crescentic basophilic mass. The nuclear wall still persists. (H) Pyknotic nucleus with dense homogenous basophilic material.



D. K. WINTER - 47

BASIC PATTERNS IN TERATOID TUMORS OF THE TESTIS *

HENRY D. MOON, M.D., and R. L. HULLINGHORST, Lt. Col., M.C., U.S. Army

*(From the Department of Pathology, University of California Medical School,
Veterans' Administration Hospital and the Letterman General Hospital,
San Francisco, Calif.)*

A series of 125 tumors of the testis was studied. Teratoid tumors comprised over 96 per cent (121 tumors) of these cases; the remainder consisted of one interstitial cell tumor, one mesothelial sarcoma of the tunicae testis, and 2 unclassified tumors. The teratoid tumors were analyzed according to basic histologic patterns. An attempt was made to correlate these patterns with the age incidence, hormonal activity, and mortality.

Included in the teratoid tumors are those commonly designated as seminomas, teratomas, embryonal adenocarcinomas, embryonal carcinomas, and chorio-epitheliomas.

HISTOLOGIC PATTERNS

Numerous preliminary surveys of all of the teratoid tumors revealed only three fundamentally distinct patterns. These were seminoma, teratoma, and carcinoma. Although deviations occurred in some of the tumors, these were considered to be of minor significance.

Seminoma Pattern

The tumor cells in the seminoma pattern were round or polyhedral and moderately large, although somewhat variable in size. The cytoplasm was abundant, and pale or clear in most instances. The nuclei were quite large, rarely multiple, had prominent nuclear membranes, and showed chromatin filaments arranged in a crosshatch pattern. The chromatin filaments often were concentrated toward the center of the nucleus. Mitotic figures were moderately numerous. The tumor cells usually were arranged in compact masses separated by fibrous connective tissue septa. There was infiltration of the fibrous septa by lymphocytes, occasionally plasma cells, and rarely multinuclear giant cells of the foreign body type. The degree of infiltration by these cells varied greatly from tumor to tumor and no line of distinction could be drawn between "seminoma with lymphoid stroma" and "seminoma without lymphoid stroma." Adjacent to the tumor, the seminiferous tubules showed varying degrees of atrophy. In some instances the seminiferous tubules were filled with large cells with clear cytoplasm closely resembling those of the tumor.

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Tumors of this pattern were apt to replace extensively the testicular parenchyma as a single lobulated mass; occasionally there were small satellite nodules. These tumors showed little tendency to invade the epididymis. Areas of necrosis often were present, and were generally more extensive in the larger tumors.

Carcinoma Pattern

The carcinoma pattern includes the types which have been called "embryonal adenocarcinoma" and "chorio-epithelioma." There was a great tendency for the embryonal adenocarcinoma and chorio-epithelioma patterns to merge. This tendency, together with the similarity in hormonal activity, makes it seem very likely that these two variants are histogenetically identical and that both arise from the chorionic plate or its derivatives.

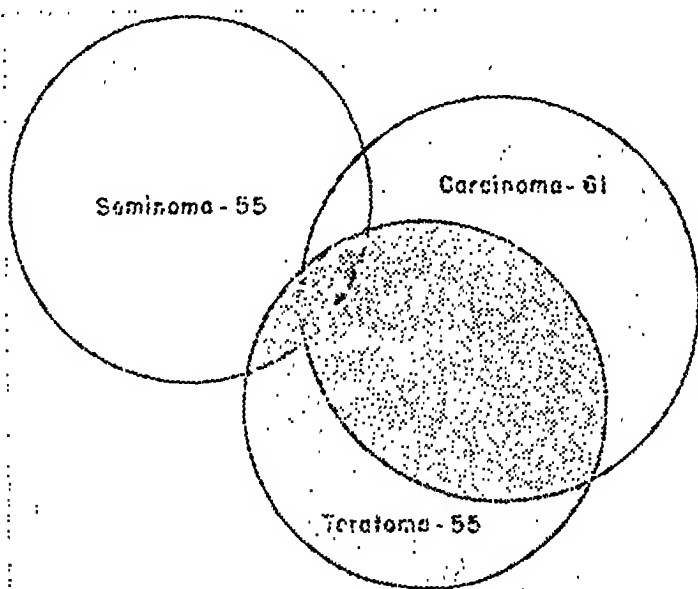
The embryonal adenocarcinoma variant was characterized by cells arranged in papillary or reticular formations, small compact groups, and perithelial clusters. Small areas of central necrosis were frequent. The tumor cells were quite variable in size but generally were larger than those in the seminoma pattern. The cell outlines usually were quite distinct. The cytoplasm varied from clear to eosinophilic and granular. The nuclei were large, with heavy nuclear membranes, and frequently were distorted and irregular; the chromatin often was clumped. A single, prominent, large, eosinophilic nucleolus was present. Mitotic figures were numerous. In many of these tumors there were focal areas showing large multinuclear giant cells (syncytial trophoblasts), in juxtaposition to smaller cells with hyperchromatic nuclei and pale cytoplasm (cytotrophoblasts). In some instances the multinuclear tumor cells were small and had pyknotic nuclei.

The chorio-epithelioma variant was characterized by masses of cytotrophoblasts and multinuclear giant syncytial cells. Occasionally, structures resembling placental villi were present. Active invasion and destruction of surrounding tissue were characteristic. Vascular invasion often was seen. Necrosis and hemorrhage occurred frequently in these tumors, and in many instances chorio-epitheliomatous elements surrounded the area of hemorrhage.

Teratoma Pattern

The teratoma pattern showed a wide range of variation from very immature embryonic structures to adult structures. At one extreme there were numerous, primitive organoid structures of epithelium and mesenchyme; at the opposite extreme there were easily recognizable, well differentiated structures such as intestinal mucosa, keratinizing

squamous epithelium, cartilage, and muscle. In some of the tumors of the teratoma pattern, structures of various developmental stages occurred together. In other tumors one element might tend to overgrow all others. Occasionally, the epithelial elements showed a tendency to dominate the picture, so that it was difficult to differentiate these areas from the carcinoma pattern. In still other tumors there were areas which appeared to be histologically malignant supporting tissue, *e.g.*, myosarcoma. No attempt was made to divide the tumors of teratoma pattern into those showing histologic malignancy and those showing histologic benignity. In our series no metastasis of only a *single* dif-



Text-Figure 1. Incidence and overlapping of basic patterns in teratoid tumors of the testis.

ferentiated tissue, such as muscle, bone, or skin, occurred; the metastatic lesions which contained tissues of the embryo proper showed the presence of differentiated tissue of more than one type.

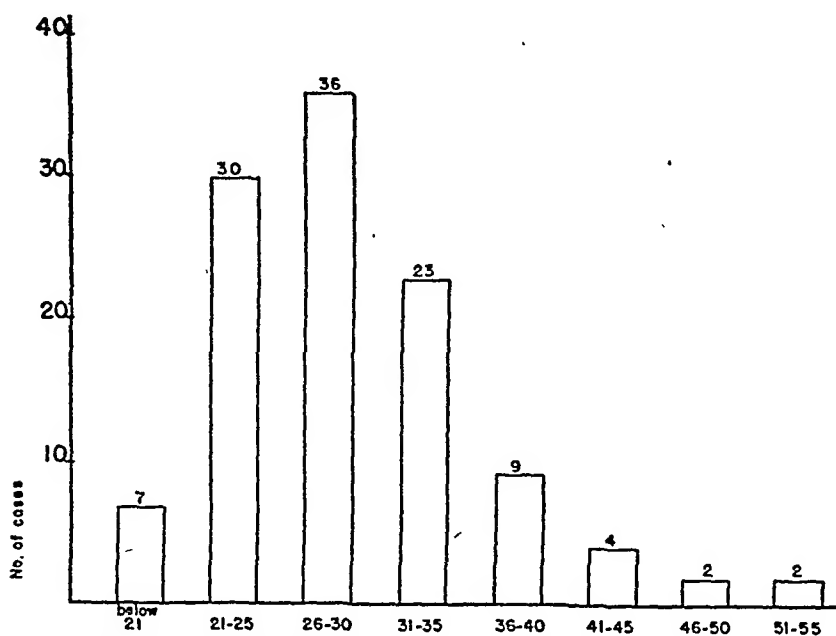
INCIDENCE AND OVERLAPPING OF BASIC PATTERNS

In the group of teratoid tumors of the testis, the three patterns of seminoma, teratoma, and carcinoma were encountered with approximately equal frequency. The seminoma pattern was encountered in 55, the teratoma pattern in 55, and the carcinoma pattern in 61 tumors. In 75 tumors a single pattern was present, and these tumors are those subsequently referred to as "pure." There were 44 pure seminomas, 13 pure teratomas, and 18 pure carcinomas. In 46 tumors various combinations of the three basic patterns were present (Text-Fig. 1). The teratoma-carcinoma combination was the one most frequently observed, and this was seen in 35 cases. The seminoma-teratoma com-

bination occurred in 3 cases, the seminoma-carcinoma combination occurred in 4 cases, and the seminoma-teratoma-carcinoma combination occurred in 4 cases. The confusing complexity of these tumors is due to a large extent to this tendency for more than one pattern to occur in the same tumor (*i.e.*, overlapping of patterns). It was felt desirable for the purposes of this study to designate a mixed or "impure" tumor by the particular patterns present.

AGE INCIDENCE

The ages of the patients ranged from 1½ to 53 years. The mean age for the entire group was 29.0 years. Over 80 per cent of these



Text-Figure 2. Age distribution of 113 teratoid tumors of the testis.

tumors occurred between the ages of 20 and 35 years. Text-Figure 2 shows the distribution of the teratoid tumors by age groups.

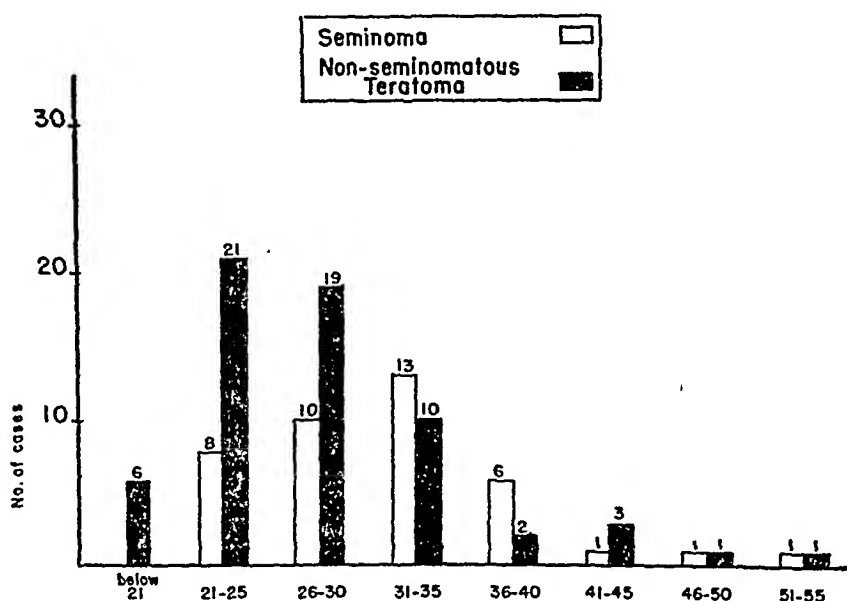
The age incidence of the tumors was studied with reference to the three basic patterns. The mean age of the pure seminomas was found to be slightly older than the mean age for all of the other teratoid tumors of the testis (Text-Fig. 3 and Table I). However, this difference was not found to be statistically significant. No significant differences could be demonstrated in the tumors of teratoma and carcinoma patterns.

HORMONAL ACTIVITY

The excretion of chorionic gonadotropin in the urine of many patients with teratoma testis is well known.¹⁻³ The production of interstitial cell hypertrophy and hyperplasia in laboratory animals by administration of chorionic gonadotropin is also well established.^{4,5}

Hyperplasia of the interstitial cells of the testis has been noted also in many of our cases of teratoid tumor of the testis.

It was thought that it would be of interest to correlate the hyperplasia of interstitial cells with the basic tumor patterns of teratoid tumors. This phase of the study was limited to those cases in which there was sufficient testicular tissue remaining to permit an evaluation



Text-Figure 3. Distribution of pure seminoma and nonseminomatous teratoid tumors of the testis according to age.

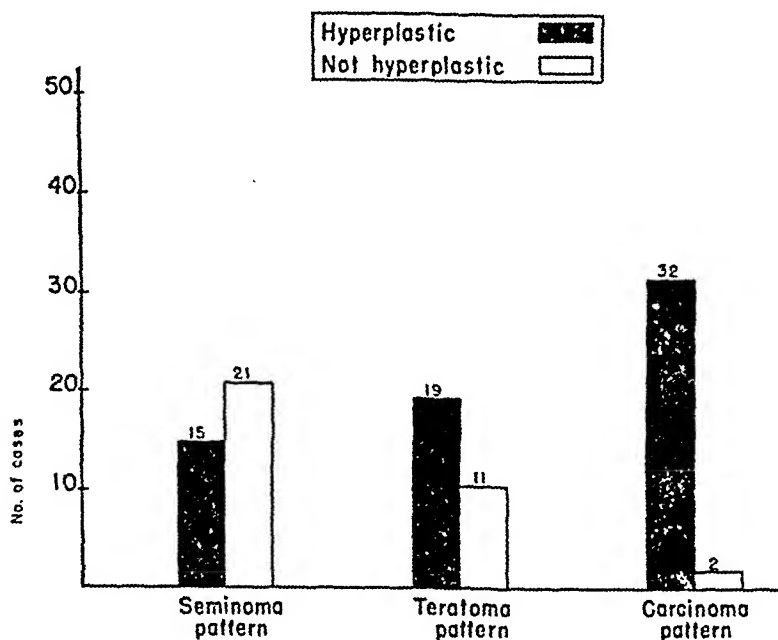
of the status of the interstitial cells. The nature of the material did not allow a quantitative analysis. For this reason, emphasis was laid on the size of the individual interstitial cells and on the size of the groups of interstitial cells. We feel that the qualitative differences which were observed are significant because there was no correlation between the amount of testicular parenchyma replaced and the degree of interstitial cell hypertrophy and hyperplasia, indicating that this is not a compensatory hypertrophy.

Of 72 tumors studied, there were 41 tumors (56.9 per cent) which were associated with interstitial cell hypertrophy and hyperplasia. The

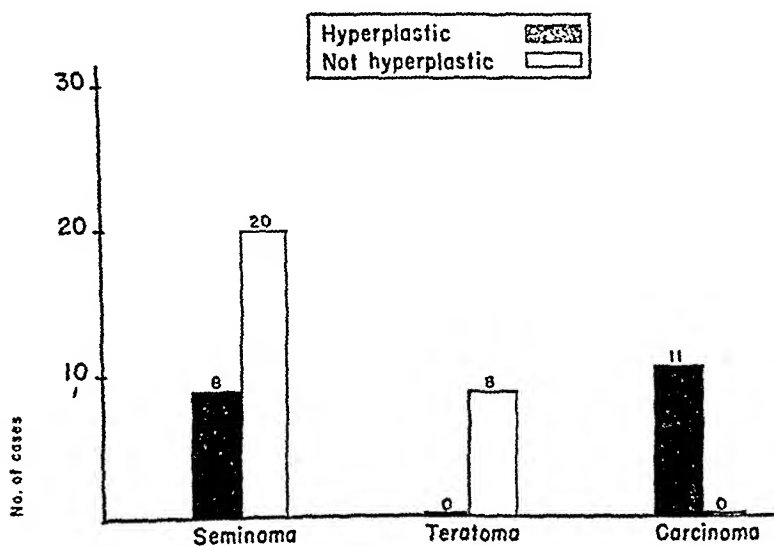
TABLE I
*Statistical Comparison of Seminomas and Nonseminomas
in Respect to Age of Patients*

	No. of cases	Range	Mean
Pure seminoma	40	years 21-53	years 31.35 ± 7.1
Teratoid tumors exclusive of those with seminoma element	63	1-53	27.74 ± 7.9

status of the interstitial cells was determined for each of the three basic patterns regardless of the presence of other patterns in the same tumor. The results of this analysis are presented in Text-Figure 4. Then the status of the interstitial cells was determined for each of the three



Text-Figure 4. Hyperplasia of interstitial cells in teratoid tumors according to pattern (including mixtures of impure tumors).



Text-Figure 5. Hyperplasia of interstitial cells in teratoid tumors with pure patterns.

basic patterns, excluding all cases which showed more than one pattern; in other words, only the pure tumors were included. The results are shown in Text-Figure 5.

The Friedman test⁶ was performed on 42 cases prior to orchiectomy. A positive reaction was obtained in 13 cases (30.9 per cent), and a negative reaction in 29 cases (69.1 per cent). In 10 cases with a

negative Friedman reaction there was hypertrophy and hyperplasia of the interstitial cells. There was no instance in which there was a positive Friedman test and absence of stimulation of the interstitial tissue. Observations on the interstitial tissue and the Friedman reactions are summarized in Table II.

TABLE II
State of the Interstitial Tissue and Results of Friedman Tests in Respect to Basic Types

	Interstitial tissue		Friedman test	
	Hyperplastic	Not hyperplastic	Positive	Negative
Pure seminoma	8	20	0	12
Pure teratoma	0	8	0	1
Pure carcinoma	11	0	5	5
Seminoma-teratoma	1	1	0	2
Seminoma-carcinoma	3	0	0	1
Teratoma-carcinoma	15	2	8	7
Seminoma-teratoma-carcinoma	3	0	0	1

MORTALITY

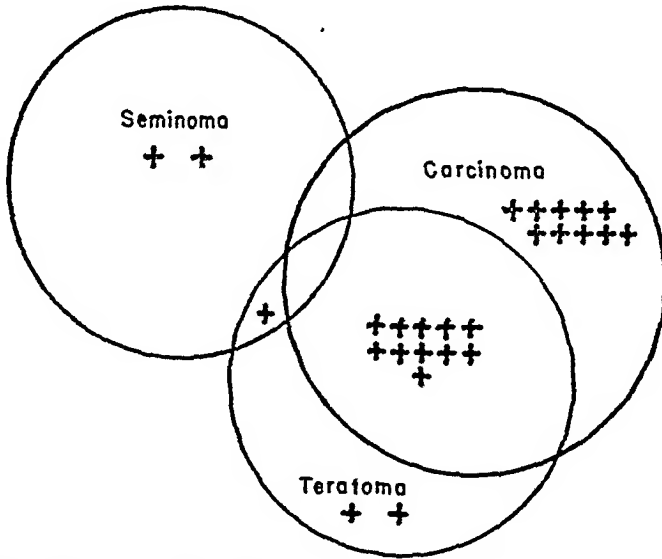
There have been 26 known deaths in this group. All but 2 have occurred within $2\frac{1}{2}$ years after the tumor was discovered. In 3 cases the presenting symptoms were those due to metastases, and in one case the testicular tumor was found only at autopsy. In the 26 fatal cases the seminoma pattern occurred 3 times, the teratoma pattern 14 times, and the carcinoma pattern 21 times. The marked tendency of the teratoma and carcinoma patterns to occur in the same tumor again was noted, and in 11 of the fatal cases this combination was present. Only 2 deaths occurred in the pure seminoma group and 2 deaths in the pure teratoma group, whereas 10 deaths occurred in the pure carcinoma group. The results show that the carcinoma pattern has a much poorer prognosis (Text-Fig. 6).

There have been no deaths reported in our series in patients in whom interstitial cell hyperplasia was absent. There were 9 deaths in cases in which the available pathologic material did not allow an evaluation of the status of the interstitial tissue. Thus, on the basis of follow-up studies to date, absence of interstitial cell hyperplasia should be regarded as a favorable prognostic sign. On the other hand, 4 deaths have occurred in cases with a negative preoperative Friedman test.

DISCUSSION

The seminoma pattern showed relatively slight tendency to occur in conjunction with other patterns. On the other hand, the combination of teratoma and carcinoma patterns in the same tumor was encoun-

tered frequently. This latter finding supports the concept that the teratoma and carcinoma patterns are the result of differentiation of a pluripotent tumor cell into tissues of two types: those corresponding to the embryo proper (teratoma), and those corresponding to the derivatives of the chorionic plate (carcinoma). The occurrence of both teratomatous and carcinomatous elements in the same metastatic lesion suggests that the malignant cell is sufficiently undifferentiated and pluripotent to allow differentiation into these various elements after localization in the secondary site. We believe that the pure teratomas and pure carcinomas represent a unilateral development of a pluripotent cell.



Text-Figure 6. Distribution of deaths according to pattern.

In some instances within the teratoma group there was marked disorientation and proliferation of neurogenic, epithelial, or mesenchymal elements, suggesting malignant proliferation of only one element in a teratoma. However, no instance was found in which there was metastasis of only one type. Invariably, metastasis in such tumors consisted of teratoid tissue of more than one type, and usually included tissue showing the typical carcinoma pattern. For this reason, no attempt was made to designate a single element of a teratoma as being malignant, such as "rhabdomyosarcoma in a teratoma."

A close correlation between interstitial cell hyperplasia and the carcinoma pattern was noted; in *all* tumors with the carcinoma pattern in which the interstitial cells could be studied, hyperplasia was found. In no instance was absence of hyperplasia noted with a positive pre-operative Friedman test. The remarkably close correlation of interstitial cell hypertrophy and hyperplasia with the carcinoma pattern, even in cases showing a negative Friedman reaction, would suggest that this finding may be used as an indication that tumor of the carci-

noma pattern is present. The occurrence of interstitial cell hypertrophy and hyperplasia in 28 per cent of the cases classed as pure seminoma suggests the presence of carcinoma patterns in these cases even though not demonstrated in the histologic sections. In 2 such cases examination of additional material resulted in the demonstration of carcinoma elements.

SUMMARY

In a series of 125 tumors of the testis, 96 per cent were found to be teratoid tumors.

Only three fundamentally distinct patterns, namely, seminoma, teratoma, and carcinoma, could be demonstrated consistently in the group of teratoid tumors. In 75 tumors only one pattern was present, whereas in 46 tumors there was more than one pattern.

The mean age for the entire group of patients with teratoid tumors was 29.0 years. The mean age for those with seminomas was 31.35 years; the mean age was 27.74 years for those with nonseminomatous tumors. This difference is not statistically significant.

Hypertrophy and hyperplasia of the interstitial cells of the testis were found to be very closely correlated with the carcinoma pattern.

In the known deaths which occurred in these cases, the carcinoma pattern was present much more frequently than the other two patterns.

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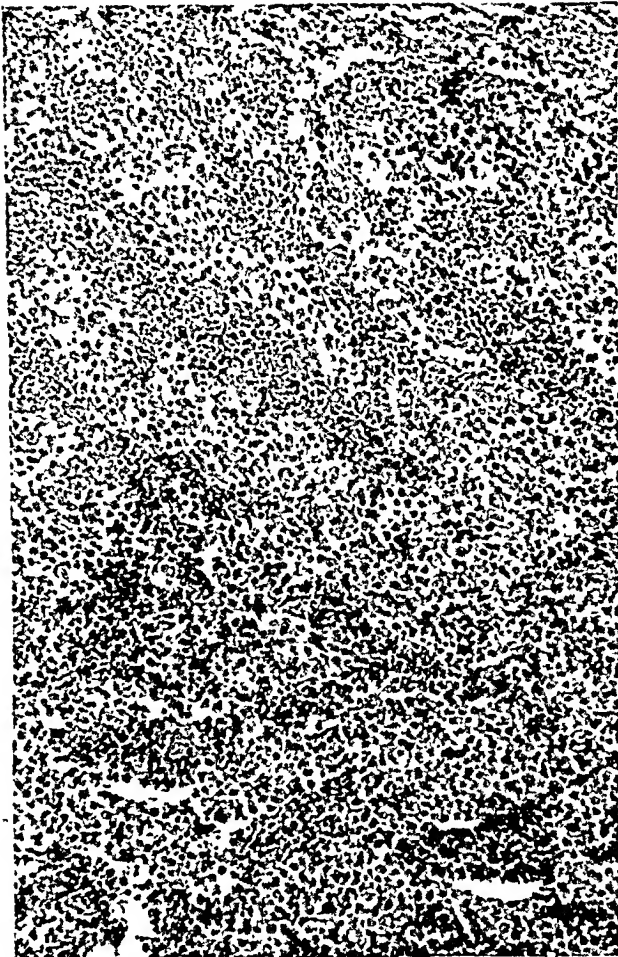
[Illustrations follow]

DESCRIPTION OF PLATES

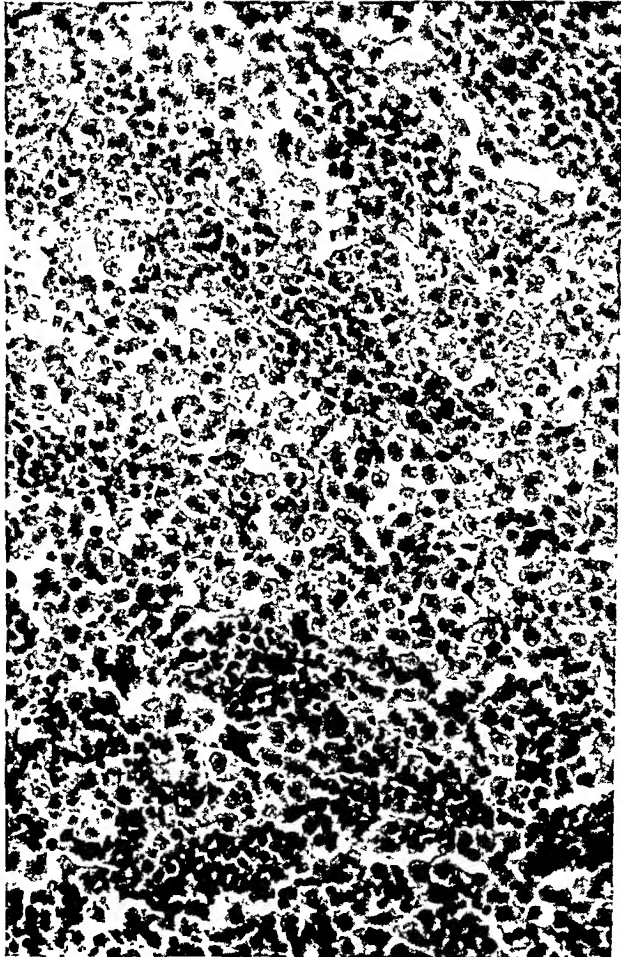
PLATE 173

- FIG. 1. Teratoid tumor of the testis of the seminoma pattern. $\times 50$.
FIG. 2. Teratoid tumor of the testis of the seminoma pattern. $\times 100$.
FIG. 3. Teratoid tumor of the testis of the seminoma pattern. $\times 50$.
FIG. 4. Teratoid tumor of the testis of the seminoma pattern. $\times 100$.

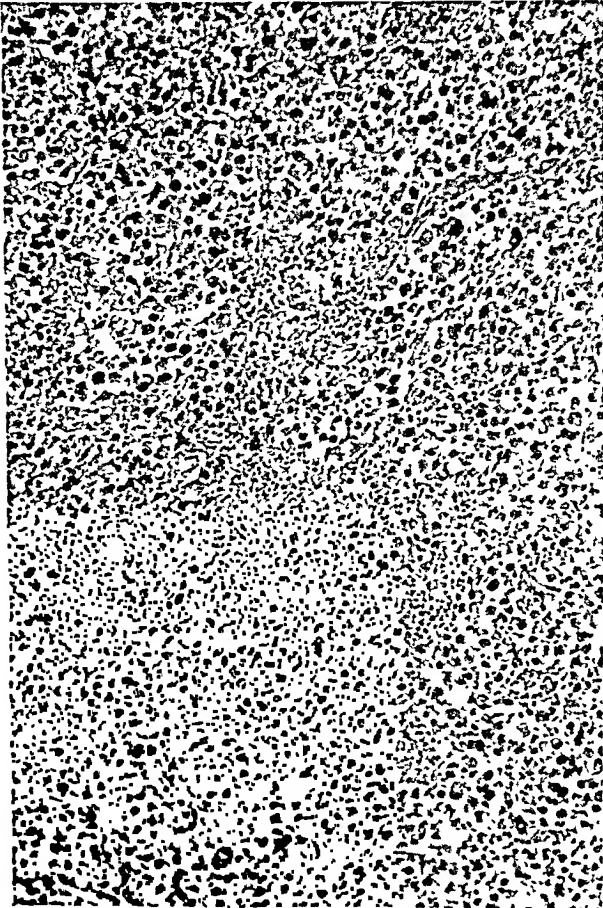
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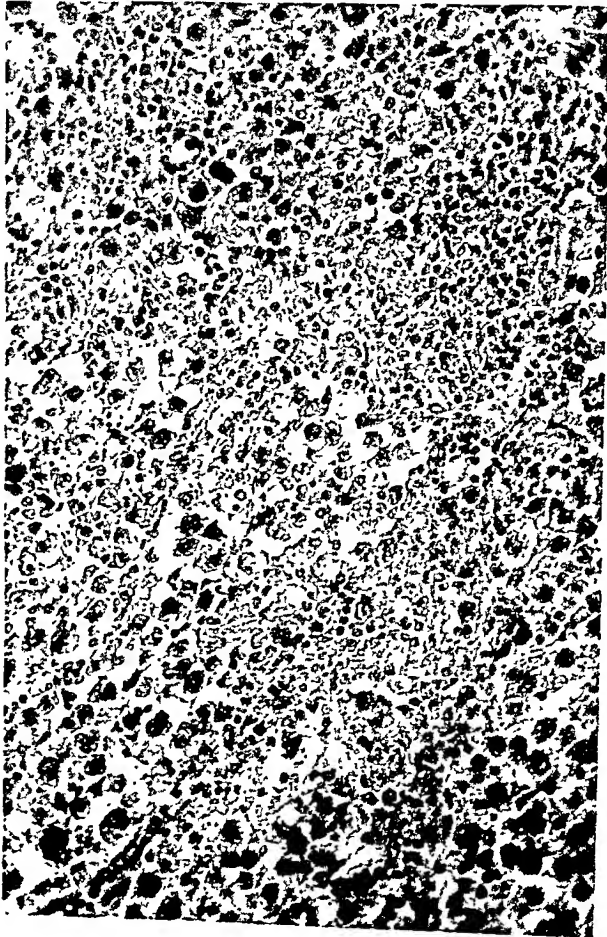


PLATE 174

FIG. 5. Teratoid tumor of the testis of the carcinoma pattern (adenocarcinoma variant). $\times 50$.

FIG. 6. Teratoid tumor of the testis of the carcinoma pattern. $\times 50$.

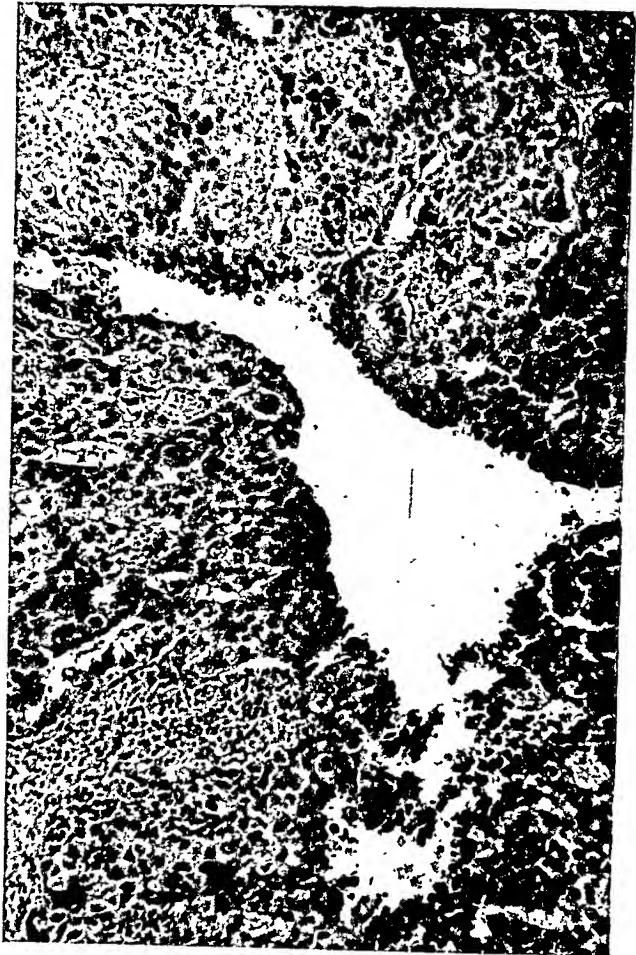
FIG. 7. Teratoid tumor of the testis of the carcinoma pattern (chorio-epithelioma variant). $\times 100$.

FIG. 8. Hyperplasia of interstitial cells associated with a tumor of the carcinoma pattern. $\times 50$.

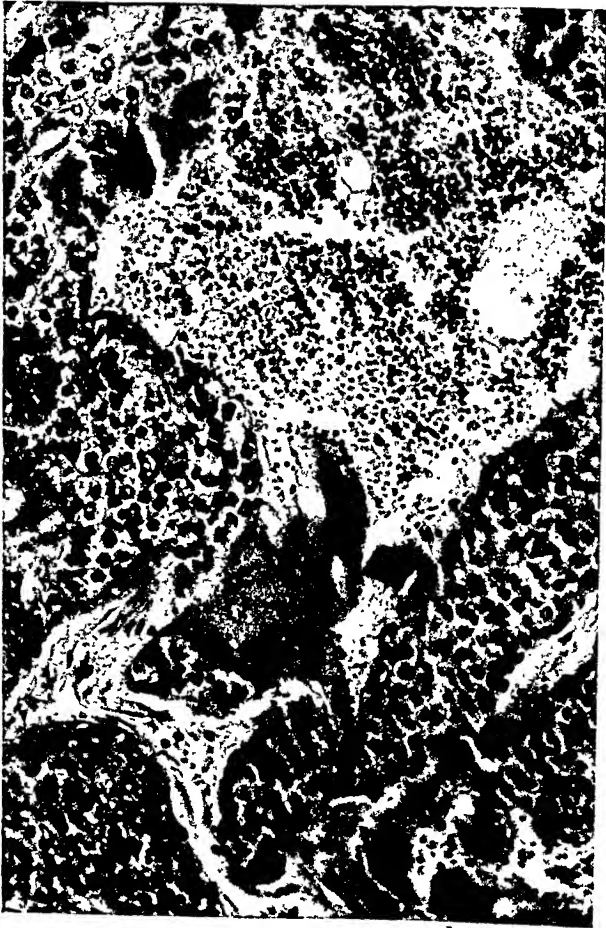
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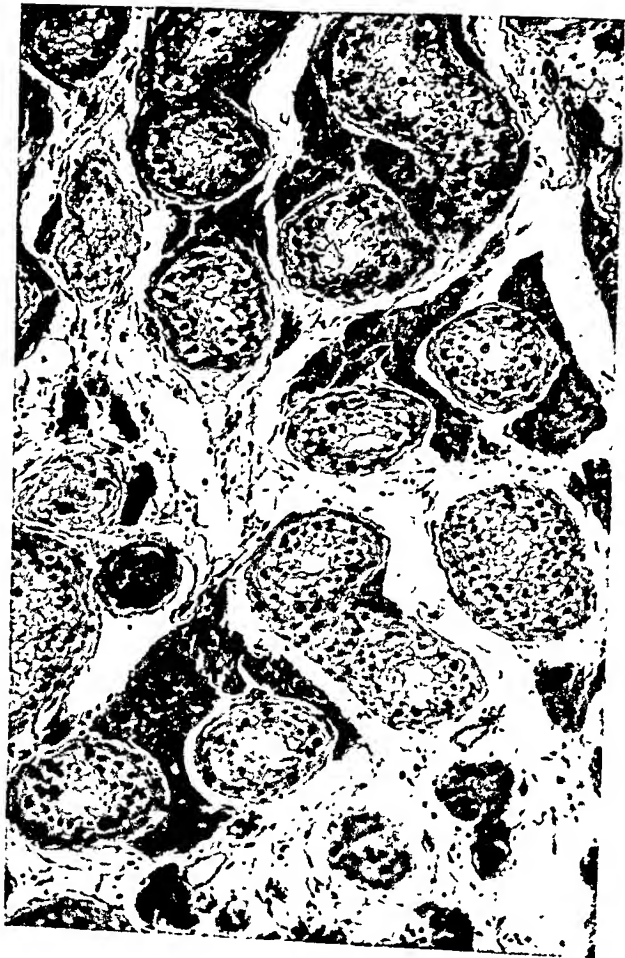
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Moon and Hullinghorst

Teratoid Tumors of the Testis

PLATE 175

FIG. 9. Testicular tumor of the teratoma pattern (embryonal variant). $\times 50$.

FIG. 10. Testicular tumor of the teratoma pattern (adult variant). $\times 50$.

FIG. 11. Combined seminoma and carcinoma patterns. $\times 100$.

FIG. 12. Combined teratoma and carcinoma patterns. Both the adenocarcinoma and chorio-epithelioma variants are present. $\times 50$.

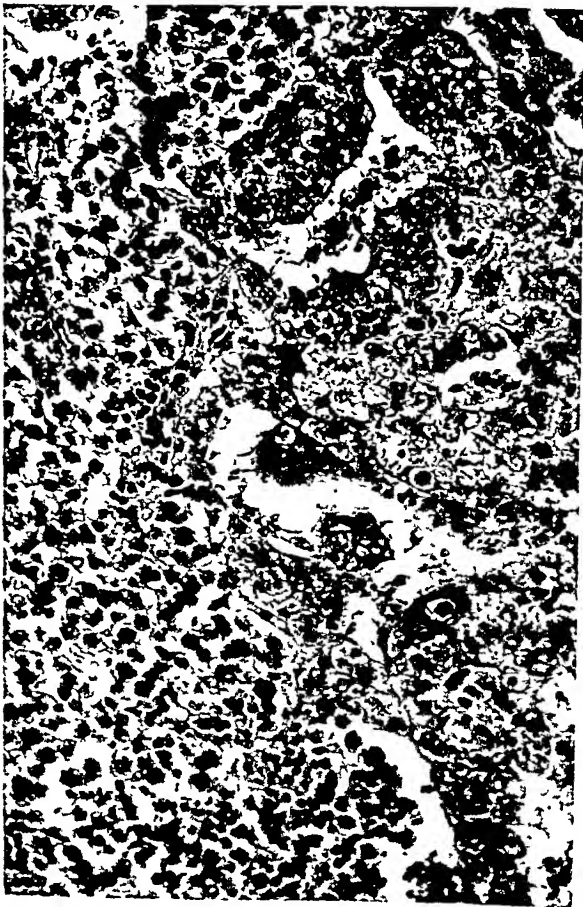
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PULMONARY ADENOMATOSIS A REPORT OF THREE CASES *

GEORGE W. DRYMALSKI, M.D., J. ROBERT THOMPSON, M.D., and HENRY C. SWEANY, M.D.

*(From the Research Laboratories of the Municipal Tuberculosis Sanitarium,
Chicago, Ill.)*

Helly,¹ in 1907, described a multiple, nodular, bilateral tumor of the lungs in a woman, 43 years of age. Because of the preservation of the alveolar septa which were lined with cylindric cells and the absence of metastases, he termed the growth an adenoma, as distinguished from an alveolar cell carcinoma.

Of considerable current interest, pulmonary adenomatosis is not without curious and speculative features. Since Helly's report, 12 cases have been described in the literature²⁻¹²; 6 of these have been reported within the past several years. The renewed interest in the subject perhaps has been stimulated by Bonne's² observations on the morphologic resemblance of pulmonary adenomatosis in sheep (*jaagsiekte*) to certain cases of pulmonary cancer in man.

Epizootic pulmonary adenomatosis of sheep is of global distribution. In 1915 Mitchell¹² extensively described the condition, and in 1938 Dungall¹³ reported an epidemic in Iceland. The usually fatal ovine disease, the lesions of which resemble pulmonary lesions found in sheep suffering from sheep-pox, consists of a certain amount of interstitial fibrosis and a tumor-like proliferation of pulmonary epithelium. It has in common with human pulmonary adenomatosis multicentricity, columnar nonciliated cells, absence of stromal invasion, and infrequent mitotic figures.¹⁰ No specific agent has been proved to be the cause of the disease, but many investigators^{2,9,14} have suspected that a virus is the inciting and responsible factor. Transmission of the disease is affected easily by housing healthy sheep with diseased sheep. However, experimental infection has been generally unsuccessful; in only one instance was the condition reproduced in a sheep by an intrapulmonary injection of a tissue suspension.¹⁵ Efforts to isolate a virus from a human specimen of pulmonary adenomatosis were not successful.⁸ On the other hand, Grumbach¹⁶ found diffuse alveolar epithelization after inoculating a guinea-pig with a diphtheroid bacillus obtained from a lymph node of a patient with Hodgkin's disease. Subcutaneous injection of 1, 2, 5, 6-dibenzanthracene into mice produced multiple malignant tumors of alveolar origin.¹⁷ Norris¹⁸ injected fluid aspirated from a patient with lobar pneumonia into a guinea-pig. One lung of the guinea-pig subsequently contained lesions resembling those of *jaag-*

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siekte in sheep. In various spontaneous pathologic conditions of the lung, epithelium-like lining cells are found along the alveolar walls,^{7,10} and it has been suggested that the difference between simple localized hyperplasia or metaplasia and adenomatosis may be more apparent than real.⁸

The evidence seems to indicate that pulmonary adenomatosis is a specific response to certain nonspecific irritants. This hyperplastic response eventually causes death by extensive involvement of lung parenchyma or by progressing into a true malignancy.

The gross pathology of pulmonary adenomatosis usually resembles pneumonia in gray hepatization. Occasionally mucus can be scraped from the cut surface. Nodular consolidated areas, which have been compared to noncaseating miliary tuberculosis, have been described. A primary bronchial focus has never been demonstrated. Nonciliated cuboidal or columnar cells, lining otherwise unaffected alveolar walls, characterize the microscopic changes. The cytoplasm of these cells is eosinophilic, granular, or somewhat foamy. Goblet formation and a brush border may be noted. The nuclei are usually basally or subcentrally located; nucleoli may be prominent and the chromatin is coarse. In general, the cells are uniform and mitotic figures are uncommon. The alveolar septa are not involved or show only slight thickening; absence of invasion is a feature of the disease. The alveolar spaces contain desquamated cells, occasional phagocytes, and a variable amount of exudate and mucus. Bronchopneumonia often obscures the significant features of the condition.

With microscopic features identical with those of the so-called alveolar cell carcinoma, pulmonary adenomatosis differs from carcinoma by virtue of its presumably nonmetastasizing nature. However, of the 12 previously reported cases of adenomatosis, 2 showed metastatic lesions.^{4,5} In one of the cases presented in this study malignant proliferation was noted (Fig. 6). Metastasis has occurred in jaagsiekte of sheep. There is no reason to assume that pulmonary adenomatosis is forever a benign growth, and it is possible that pneumonia, which usually complicates the disease, may cause death before metastasis can develop.¹¹ It is likely that adenomatosis of the lungs is a relatively benign variant of the so-called alveolar cell carcinoma and is potentially capable of developing all of the characteristics of malignancy. We feel relatively certain that a case of so-called alveolar cell carcinoma previously reported from this laboratory was actually a case of pulmonary adenomatosis.²⁰ Of academic interest and of great controversy is the cellular origin of these tumors. Theoretically, the cells may be derived from bronchiolar or alveolar epithelium. Loosli²¹

and others ^{22, 23} have maintained that no true alveolar lining exists and that the capillaries are contained in a ground substance with infrequent cells of mesenchymal origin. Other workers ^{24, 25} believed that the alveoli have a continuous epithelial lining. Because of the decided epithelial nature of the adenomatous cells and, more particularly, the absence of cilia, either the lining cells of the alveoli or the lining cells of the terminal bronchioles are the cells of origin of these tumors. Since the number of those who deny the existence of alveolar epithelium is increasing because of inability to demonstrate the cells,²⁶ the possibility of a bronchiolar origin probably should be given preferential consideration. In one of the cases presented in this report (Figs. 2 and 3), several terminal bronchioles with normal epithelium that abruptly shifted to columnar adenomatous cells were seen.

It is unfortunate that there are no signs or symptoms pathognomonic of pulmonary adenomatosis. Radiologically, the condition simulates tuberculosis, carcinoma, or pneumonia. The patients give no history of exposure to sheep. Dyspnea and a cough productive of mucoid sputum are prominent inaugural symptoms. Statistics on bronchogenic carcinoma show a ratio of 3 men to 1 woman; in the so-called alveolar cell carcinoma the ratio is 1:1. In the collected cases of pulmonary adenomatosis, including those reported here, there were 9 women and 6 men. Their average age was almost 54 years. The duration of the disease has varied from several weeks to over 2 years. The incidence of pulmonary adenomatosis in our series of 57 cases of lung carcinoma is 5 per cent. Neuburger and Geever²⁶ also estimated the incidence of alveolar cell carcinoma as 5 per cent.

REPORT OF CASES

Case 1

H. D., an unmarried woman of Polish ancestry, 34 years old, had lived in Chicago all of her life. She had been a hospital attendant for 10 years and there was some contact with tuberculous patients. Admitted to the Municipal Tuberculosis Sanitarium on July 1, 1946, her symptoms consisted of a nonproductive cough and a weight loss of 4 lb. A routine roentgenogram of the chest a few months previously had disclosed a 3 cm. cavity in the right lower lobe. Her general health was good.

On physical examination, abnormal findings were limited to the chest. There was slightly impaired expansion with harsh breath sounds in the right lower lobe anteriorly. The vital capacity was 2600 cc. There was no fever. A bronchogram revealed no abnormalities. The Mantoux test was positive, and all sputum examinations were negative for tubercle bacilli. Staphylococci were cultured from the sputum; there was no anaerobic growth. The red blood cell count was 4.2 million, the hemoglobin was 69 per cent. The white blood cell count was 4,900 with 46 per cent polymorphonuclear leukocytes, 39 per cent lymphocytes, and 14 per cent monocytes. Kahn test of the blood was negative, and the urinalysis was normal. Bronchoscopy was negative except for signs of chronic suppuration of the right lower lobe.

The patient was given 1 million units of penicillin. There was some clearing of the soft infiltrate about the cavity wall as determined roentgenologically. She was discharged on October 30, 1946, with a diagnosis of cavity of the right lower lobe of undetermined origin.

On January 9, 1947, a roentgenogram of the chest showed no changes in comparison with previous films.

On June 3, 1947, the patient was readmitted. During March and April she had suffered two attacks of bronchopneumonia with a fever up to 105° F., hemoptysis, and severe pain over the right lower chest. On examination there was increased vocal fremitus over the right lower lobe with dullness, bronchial breathing, and amphoric breath sounds posteriorly. Some consonating râles were heard in this area. A roentgenogram showed consolidation and atelectasis of the right lower lobe. Total blood protein was 8.5 gm., and the white blood cell count was 6,200 with 64 per cent polymorphonuclear leukocytes. Other laboratory findings were unchanged from those of the previous admission.

A lobectomy was performed by Dr. Richard Davison on June 19, 1947. The preoperative diagnosis was chronic lung abscess with pneumonitis and bronchiectasis. The postoperative course was uneventful.

On gross examination, the right lower lobe of the lung was firm throughout. There were a few thin, fibrous tags on the pleural surface. On section, the parenchyma was uniformly solid and resembled the gray hepatized stage of pneumonia. There was no mucous exudate. At the apex of the lobe was a small (2 cm.), irregular cavity with ragged walls and an absence of exudate. Microscopically, there were tall, thick, nonciliated columnar cells which lined the alveolar walls. The cytoplasm was eosinophilic and slightly granular. The nuclei were regular in size and basally situated. Mitotic figures were uncommon. Papillary formation was noted frequently and the alveoli contained a few desquamated columnar cells, occasional polymorphonuclear leukocytes, and a slight amount of mucus. In the area of ulceration there was marked fibrous hyperplasia with degeneration of the adenomatous cells. Elsewhere the alveolar septa were intact. A bronchiole showed transformation of the normal mucosa to tall-columnar nonciliated cells (Fig. 2). No tumor cells were evident in the pleura, the lymph spaces, or in the blood vessels. The bronchial lymph nodes were not dissected at the operation.

Case 2

N. D., a white woman, 55 years of age, developed a mild cough in August, 1939. Pulmonary tuberculosis was diagnosed by her private physician after roentgenologic examination of the chest. She entered the Municipal Tuberculosis Sanitarium in December, 1939, complaining of a cough and a small amount of sputum which was occasionally streaked by blood. Fatigue and weakness were noted.

On physical examination the patient was found to be in good general condition. The blood pressure was 160/100 mm. Hg. Expansion of the chest was limited and resonance was impaired throughout the right upper one-third and throughout the entire left hemithorax. Coarse breath sounds with scattered moist râles were heard in these areas. A roentgenogram revealed areas of marked haziness in both lungs, most pronounced in the right. The cardiac shadow was displaced to the left.

All sputum examinations were negative for tubercle bacilli. *Streptococcus*

viridans was cultured from the sputum. The hemoglobin was 85 per cent; white blood cell count, 9,250 with 66 per cent neutrophils. The sedimentation rate was 6 mm. in 30 minutes (Cutler). The Mantoux test was positive and Kahn test of the blood was negative. The urinalysis gave normal findings. A sputum section on January 14, 1940, showed many epithelial cells which were single or in small groups. These cells were ovoid with round, vesicular nuclei and eosinophilic cytoplasm. Some nuclei were hyperchromatic. The diagnosis was pulmonary carcinoma. An aspiration biopsy of the lung a few days later was diagnosed as squamous cell carcinoma.

The patient became progressively more dyspneic and weaker. Death occurred on February 16, 1940.

Autopsy was performed 9 hours after death; permission was granted to remove only the lungs. A thin deposit of fibrin covered each lung and there were multiple adhesions throughout. Moderately distended and slightly anthracotic, both lungs on section contained several firm, dull gray areas in the mid-zones. Round, rather sharply defined and occasionally confluent, these lesions were similar in appearance to gray pneumonic hepatization. In the right base a firm gray nodule was found beneath the pleura. Except for edema the remaining lung fields were uninvolved. A frothy fluid filled the bronchi. The hilar lymph nodes were of normal size and contained no tumor metastases.

Microscopically, the septa were lined by high, nonciliated columnar cells with abundant eosinophilic cytoplasm and an occasional brush border. The nuclei were oval, situated basally, uniform in size, and somewhat vesicular. Papillary formation was pronounced, but mitotic figures were infrequent. There was slight thickening of the alveolar septa. Disseminated throughout was an acute necrotizing bronchopneumonia with marked inflammatory exudation and infiltration. A fibrinous pleuritis was extensive. Attended by necrosis, organization and early fibrosis, the adenomatous cells in one small area had lost all attributes of differentiation (Fig. 6). Anaplastic, invasive, and destructive, the tumor cells were irregular in size and shape and mitotic figures were common. The anatomic diagnosis was pulmonary adenomatosis with malignant degeneration.

Case 3

C. Z., a white housewife, 45 years old, was admitted to the sanitarium on October 2, 1934. Two years previously she had suffered an attack of influenza of 3 weeks' duration which left her fatigued. After the discovery of tubercle bacilli in the sputum and roentgenologic evidence of a pulmonary lesion, pneumothorax was established in May, 1933. Because of progressively severe dyspnea, the pneumothorax was discontinued after 1 year. In August, 1934, she suffered a pulmonary hemorrhage of 10 oz. Since the onset of her illness there had been an intermittent cough which was usually nonproductive. Weight loss was 25 lb. Her appetite was poor and her strength was failing.

Physical examination revealed a woman in fair general condition. The fingers were clubbed. The blood pressure was 112/70 mm. Hg. Chest expansion was

impaired bilaterally and vocal fremitus was increased over the right base. Throughout the right chest and over the lower one-half of the left, resonance was impaired. Breath sounds were harsh in these areas. Posttussive râles were heard throughout the right lung. Roentgenograms showed marked hazy infiltration of the right lung and of the middle field of the left lung. Bronchoscopy and esophagoscopy revealed no abnormalities. The red cell count was 4.2 million with 70 per cent hemoglobin. The white blood cell count was 10,600 with 85 per cent polymorphonuclear leukocytes. Kahn test of the blood was negative. No malignant cells were found in a sputum section and repeated sputum examinations were negative for tubercle bacilli. The urinalysis was negative.

In spite of bed rest the dyspnea continued. The diagnosis was far advanced tuberculosis, although bronchogenic carcinoma was considered. The patient died on March 8, 1935.

Autopsy was performed 6 hours after death. The right pleural space was completely obliterated by fibrous adhesions which were most marked in the right upper lobe. The entire right lung was consolidated and on section was gray and hepatized. In the left lung a similar lesion fanned out from the hilum and reached the pleura laterally and the base posteriorly. The bronchi and pulmonary lymph nodes were normal. Other autopsy findings were not related to the pulmonary disease.

Microscopically, much of the lung tissue was replaced by areas of large, irregular, branched alveoli which were lined by cuboidal cells (Fig. 4). The nuclei of these cells were round, basophilic, and sometimes hyperchromatic. The cytoplasm was eosinophilic. While occasional columnar cells were seen, there was little papillary formation. Mitotic figures were rare. The interstitial tissue was much thickened by loose connective tissue and there was much proliferation of lymph vessels. There was no necrosis and the pleura was not infiltrated. An old encapsulated caseous lesion without signs of perifocal inflammation was found.

SUMMARY

Three cases of pulmonary adenomatosis are presented in this report. Case 1 is the second recorded surgical specimen of this condition and indicates a possible bronchogenic origin for the disease. Case 2 illustrates malignant proliferation of the cells of the adenomatous process. The cuboidal epithelium lining the air sacs and the loss of normal pulmonary architecture in case 3 represent an unusual variety of adenomatosis of the lung.

Representing about 5 per cent of all pulmonary tumors, adenomatosis of the lungs resembles the so-called alveolar cell carcinoma in all respects.

Probably the result of nonspecific irritation, the hyperplastic reaction of pulmonary adenomatosis is aggressive and may develop all the characteristics of malignancy.

There are no distinguishing clinical features of pulmonary adenomatosis. The average age of the patient is about 54 years, and the ratio of women to men is 3:2. The course of the disease varies from several weeks to 2 years.

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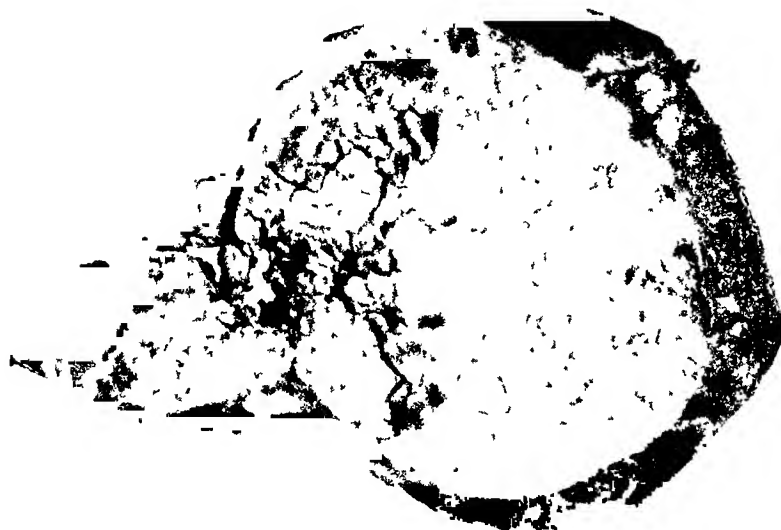
DESCRIPTION OF PLATES

PLATE 176

FIG. 1. Case 1. Photograph of the right lower lobe showing ragged ulceration and gray consolidation.

FIGS. 2 and 3. Case 1. Photomicrographs showing abrupt transformation of bronchiolar epithelium to nonciliated columnar cells and adenomatous cells lining the unaffected alveolar walls. $\times 300$ and $\times 150$.

1



2



3

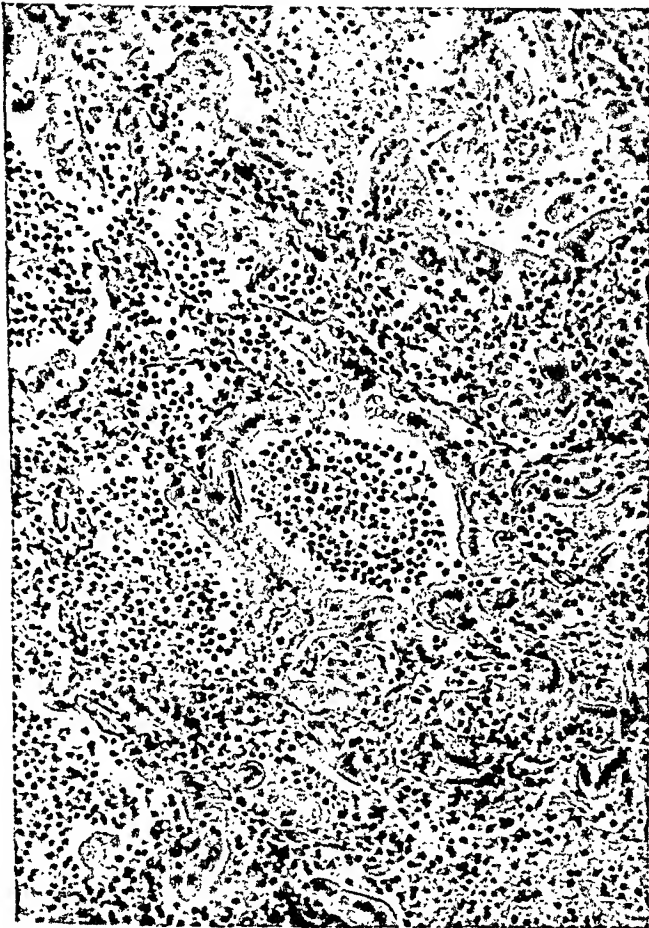
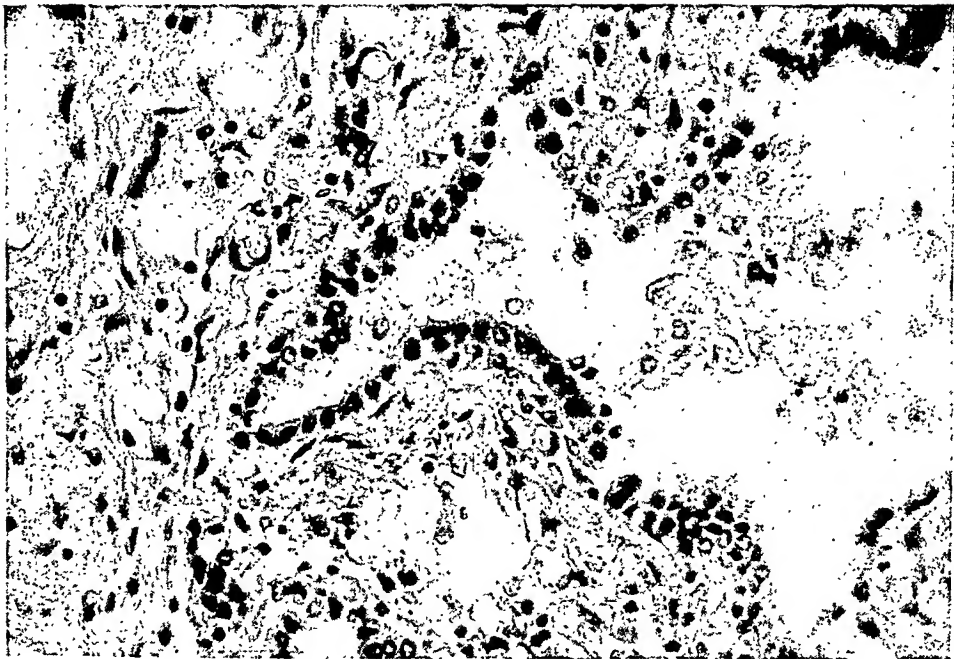


PLATE 177

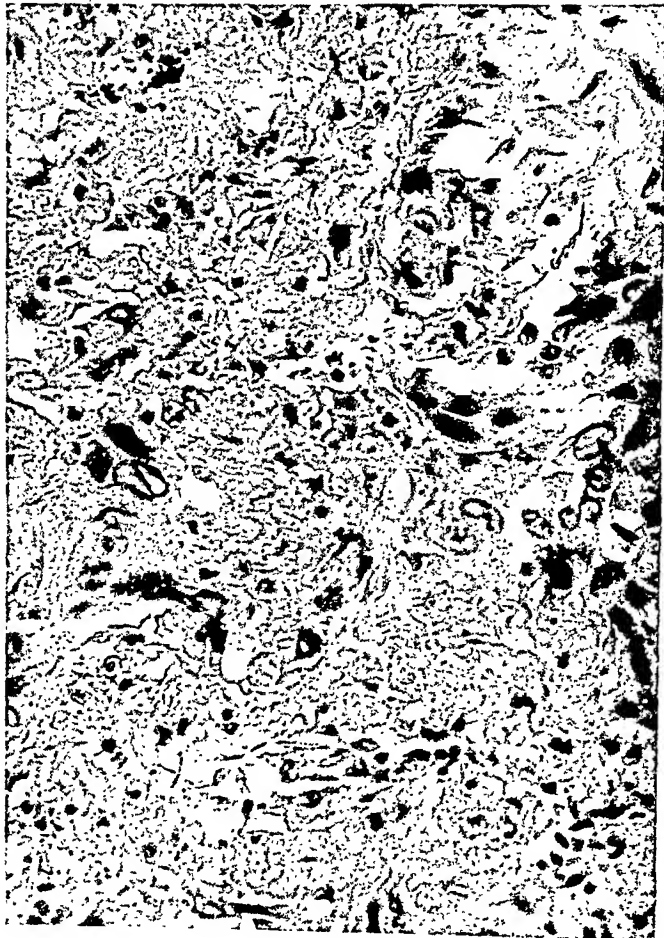
FIG. 4. Case 3. Photomicrograph showing cuboidal cells lining the air spaces and interstitial fibrosis. $\times 300$.

FIGS. 5 and 6. Case 2. Photomicrographs showing columnar cells together with pneumonic exudate and the focus of malignant proliferation found in the same lung. $\times 150$ and $\times 300$.

4



5



6



ATYPICAL AMYLOID DISEASE, WITH OBSERVATIONS ON A NEW SILVER STAIN FOR AMYLOID *

LESTER S. KING, M.D.

(From the Laboratory of the Illinois Masonic Hospital, and the Department of Pathology, University of Illinois College of Medicine, Chicago, Ill.)

There has been no general agreement on the classification of amyloid disease. Not only is there a bewildering array of names, such as primary, secondary, atypical, local, systemic, tumor-forming, and par-amyloidosis, but there is no uniformity in regard to the meaning or scope of these terms. One of the most interesting manifestations of the disease has been described as primary amyloidosis, a term especially popular in the American literature, indicative of no known cause or pre-existing disease which might be held responsible for the amyloid deposition. The two most complete reviews of the subject are the papers of Koletsky and Stecher¹ and, more recently, of Lindsay.² Koletsky and Stecher listed 22 cases, as of 1939, while Lindsay in 1946 found 45. Several other reports are mentioned by Lindsay but not considered acceptable by him. In the absence of satisfactory criteria, it may be stated that approximately 50 cases are described in the literature.

The purpose of the present communication is threefold: To present 6 cases of unusual amyloid disease, 5 of which appear similar to the rare primary form; to describe a new silver stain for amyloid; and to analyze the confusing nomenclature and terminology, and suggest a new classification.

REPORT OF CASES

(Case 1 is from the Fairfield State Hospital, Newtown, Connecticut, obtained through the courtesy of Dr. W. F. Green, Superintendent. The other 5 cases are from the Illinois Masonic Hospital, Chicago, Illinois.)

Case 1

The patient was a white male, 88 years old. He was a pauper, brought to the Fairfield State Hospital showing marked mental deterioration in all spheres. The heart sounds were of poor quality, and severe peripheral vascular sclerosis was present. The blood pressure was 140/70 mm. Hg. Moderate tremor of hands and feet was noted. Physical examination was otherwise negative. The psychiatric diagnosis was psychosis with cerebral arteriosclerosis. Laboratory data showed negative serologic findings, a red blood cell count of 4,400,000; 81 per cent hemoglobin, and a white blood cell count from 8,600 to 16,400, with a normal differential. The urine revealed occasional casts and a 1 plus albumin, otherwise negative. The nonprotein nitrogen of the blood was 28 mg. per cent. The patient succumbed to bronchopneumonia 3½ months after admission.

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The final pathologic diagnoses were: Senility; senile plaques in brain; fibrosis of spleen, pancreas, and testes; brown atrophy of liver; amyloid in heart; bronchopneumonia; fibrinous pleuritis; acute passive congestion of liver; fibrous perisplenitis; accessory spleen.

The heart weighed 350 gm. Slight calcification was present at the bases of the aortic cusps, but the free margins were delicate. No gross scarring was observable. The coronary arteries, although tortuous, revealed only mild atheromatous deposits. The aorta was the seat of only slight atheroma. Arteriosclerosis was not considered as a significant part of the disease picture. The microscopic examination of the heart is discussed later. No amyloid was noted in any other organ. The other diagnoses need no comment.

Case 2

An 83-year-old man was admitted in a somewhat disoriented condition with a history of urinary difficulty, consisting of nocturia, difficulty in starting the stream, and dribbling, of uncertain duration. On physical examination the significant findings were some increased resonance in both lungs, moderate kyphosis, and scattered râles in the right lung. The heart was moderately enlarged to percussion, and a soft systolic murmur was present at the apex. The prostate was enlarged, symmetric, firm, nontender, and smooth. The blood pressure was 140/80 mm. Hg. Slight pitting edema of the lower extremities was present. Urine showed 3 plus albumin, no sugar, and many pus cells. The red blood count was 3,620,000, with but 38 per cent hemoglobin; the white blood cell count was 9,720, with a normal differential. Nonprotein nitrogen of the blood was 47 mg., creatinine 1.6 mg. per cent. The heart action was irregular. The electrocardiogram showed auricular fibrillation and diffuse myocardial damage. There was roentgenologic evidence of Paget's disease, involving the skull, spine, femora, and pelvis. The alkaline phosphatase value was 9.8 Bodansky units and the acid phosphatase, 1.6 units. The phosphorus was 3.8, and the calcium 7.5 mg. per cent. The patient was given numerous transfusions. Following these the red blood cell count was 4,000,000; the color index, 0.8. At operation, an extensive carcinoma of the bladder, of papillary type, was excised, and the prostate removed. Pathologic examination showed benign hyperplasia of the prostate, and papillary carcinoma of the bladder, transitional cell type. The patient did not do well after operation. His nonprotein nitrogen gradually rose to 172 mg. per cent with creatinine of 5, and he expired 14 days after operation.

The pathologic diagnoses following autopsy were: Recent operation (prostatectomy and removal of carcinoma of bladder); necrotizing cystitis; chronic pyelonephritis; generalized arteriosclerosis; calcific aortic sclerosis (Mönckeberg type), mild; myocardial hypertrophy and fibrosis; amyloid in heart; chronic passive congestion of liver; congestion and edema of lungs; Paget's disease of bone.

The heart weighed 470 gm. and was dilated in all chambers. Considerable calcification was present in the sinuses of Valsalva, and in the bases of the aortic cusps and mitral ring. The free margins of the valves were delicate. Extensive calcification and arteriosclerosis were

present in the coronary vessels, as well as in the aorta and major arteries. Microscopic study of the heart is discussed later. The left kidney weighed 70, the right, 180 gm. Both were coarsely granular, with obscuration of the corticomedullary junction, with severe fibrosis, and interstitial infiltrations of lymphocytes and plasma cells. Some acute inflammatory changes were present in collecting tubules and pelvic mucosa, but these were relatively slight. The skeletal changes were typical of Paget's disease but relatively inactive. No amyloid was noted except in the heart.

Case 3

The clinical record on case 3 is inadequate. The patient, an irrational 87-year-old woman, died 30 hours after admission. On entry her abdomen was markedly distended, the legs were edematous. Temperature on admission was 97° F. The clinical impression was arteriosclerotic heart disease. The red blood cell count was 4,900,000; hemoglobin, 14.1 gm.; white blood cell count 23,250, with 91 per cent polymorphonuclear leukocytes. Serologic findings were negative. Nonprotein nitrogen of the blood was 57; urea nitrogen, 32 mg. per cent. Paracentesis was performed and approximately 500 cc. of turbid fluid were removed. The patient remained irrational. Her temperature never rose above 97° F. and was recorded at 96° F. shortly before death.

The final pathologic diagnoses were: Generalized arteriosclerosis; calcific aortic sclerosis (Mönckeberg type); acquired bicuspid aortic valve; cardiac hypertrophy and dilatation; myocardial fibrosis; amyloid in heart; chronic passive congestion of lungs; amyloid in lungs (slight); pleural effusions; cardiac cirrhosis of liver; edema of ankles; infarct of spleen; generalized peritonitis, cause undetermined; slight bronchopneumonia; transitional cell carcinoma of vagina.

The two major disease sequences could not be brought into any definite relationship. The peritonitis was generalized and severe, but no cause therefor could be ascertained. The gastro-intestinal and genito-urinary tracts were anatomically intact. No primary focus of infection could be identified. The bronchopneumonic patches in the lungs did not appear antecedent or causal to the peritonitis. The splenic infarct was bland, and not septic. It was suggested that perhaps, in the patient's enfeebled condition, peritoneal infection might have occurred from the lumen of the bowel through an anatomically intact wall.

The circulatory system proved of great interest. The heart weighed 630 gm. The aortic valve was the seat of severe calcific sclerosis, most pronounced at the bases of the cusps. A bicuspid valve was produced. The free margins of the leaflets were pliable, although slightly thickened. Calcareous deposits at the bases of the mitral cusps also were present. Fine grayish streaking was visible in the myocardium. Microscopic findings are discussed later. The findings in liver and lungs were

indicative of long-continued cardiac insufficiency, with prominent chronic passive congestion, reaching the intensity of cardiac cirrhosis in the liver. In the lung small amounts of amyloid were noted in the thickened alveolar walls, but none was seen in any other organ. A small, recent, bland infarct of the spleen was considered to be arteriosclerotic in origin. The kidneys weighed about 120 gm. each and appeared excellently preserved.

An incidental finding was a small nodule, about 1.5 by 1 cm., situated in the posterior vaginal wall at the posterior fornix, which on section proved to be a papillary carcinoma of transitional cell type. It was superficial and did not invade the vaginal wall.

Case 4

The patient was a male, 93 years old, who had had two hospital admissions. On the first his complaints were dyspnea on exertion for about 3 weeks, progressive weakness for 3 months, and black stools for 2 weeks. The significant physical findings were: Enlargement of the heart to the left, with extrasystoles and a harsh apical systolic murmur. The blood pressure was 150/80 mm. Hg; red blood cell count, 2,550,000, with 43 per cent hemoglobin; white blood cell count, normal. Nonprotein nitrogen of the blood was 47 mg. per cent, but creatinine and sugar were normal. The electrocardiogram was interpreted as showing severe myocardial damage. Stool examinations showed occult blood. After repeated blood transfusions a gastro-intestinal x-ray series was made, showing an apparent gastric ulcer on the lesser curvature. The patient's condition improved after appropriate therapy, and he was discharged 17 days later. The clinical impression was: Bleeding peptic ulcer of stomach; organic heart disease with mitral insufficiency and mild congestive failure.

The patient was re-admitted 7 weeks later because of pain, redness, and swelling of the left leg, of 3 weeks' duration. He had been relatively well on digitalis in the interim. Examination showed thrombophlebitis of the left leg; slight auricular fibrillation; blood pressure, 150/76 mm. Hg; red blood cell count, 4,700,000, with 70 per cent hemoglobin; white blood cell count, 16,500, with 85 per cent polymorphonuclear cells. Nonprotein nitrogen of the blood was 24 mg. per cent. The patient was treated with dicumarol, the prothrombin time being regulated at 35 to 40 per cent of normal. The thrombophlebitis showed marked improvement. However, 13 days after the final admission, the patient died rather suddenly.

The pathologic diagnoses after autopsy were: Generalized arteriosclerosis; myocardial fibrosis; myocardial hypertrophy; amyloid in heart; dilatation of heart; chronic passive congestion of lungs; amyloid in lungs, slight; thrombophlebitis, left femoral vein; infarct of spleen; petechial hemorrhages of intestinal serosa and pelvis of kidney. No residuum of a peptic ulcer was encountered. No pulmonary embolus was present.

The heart weighed 420 gm. Considerable epicardial fat was noted. There was moderate dilatation of the chambers and some hypertrophy of the musculature. The valves were delicate, except for slight nodular thickening of the free edges of the mitral cusps, but the chordae ten-

dineae were delicate and inserted normally. The coronary arteries, patent throughout, were tortuous, but showed no calcification and only slight atheroma. Notation was made that they appeared extraordinarily well preserved for a man of this age. Dense fibrosis was noted at the tips of the papillary muscles, and a few minute, translucent, grayish dots and fine streaks were observed in the myocardium, especially of the septum. The aorta showed mild sclerotic changes. The microscopic findings in the heart are described later. The left femoral vein was occluded by a thrombus, and chronic inflammatory infiltrations were present in the thickened wall. The splenic infarct was small, measuring only 1.5 cm. across, and was recent. Moderate chronic passive congestion involved the lungs. Small traces of amyloid were seen in the thickened alveolar walls, but not in any other organ. The petechial hemorrhages of the intestinal serosa and renal pelvis were apparently agonal.

Case 5

The patient was a woman, 88 years old, who was admitted following a fall at home, in which she fractured her right hip. The fracture was inter-trochanteric, and comminuted. Physical examination otherwise was not remarkable, except for an apical systolic murmur. With the extremity in traction, the patient was placed on sulfonamide therapy and her general progress was satisfactory. The terminal episode, 3 months after admission, was ushered in by a sudden hemorrhage from the rectum. In spite of supportive therapy, she died within a few hours.

The pathologic diagnoses were: Generalized arteriosclerosis; occlusion of inferior mesenteric artery; infarction of distal transverse colon, sigmoid, and rectum; nephrosclerosis, arteriosclerotic type; old healed infarct of lung; amyloid in heart and lung; fibrous perisplenitis; portal cirrhosis of liver, mild; bullous emphysema, moderate; atelectasis, right middle and lower lobes; lymphocytic infiltration of adrenals; parovarian cysts, bilateral; old fracture of the right hip.

The heart weighed 230 gm. Fine grayish streaks were noted in the myocardium. The aortic valve showed slight calcareous deposits at the base of the cusps. The coronary vessels were severely sclerotic, but the lumina were only moderately narrowed. The microscopic findings are considered below. The aorta revealed extensive ulceration and calcification, and the ostium of the inferior mesenteric artery was occluded. The other major arteries were affected by severe atherosclerosis and calcification. The infarction of the distal colon, sigmoid, and rectum was characteristic. The lungs, with senile emphysema and some bullous formation, weighed only 190 and 150 gm. respectively. Some atelectasis also was present. One minute area of fibrosis, with fibrotic occlusion of a nearby artery, was interpreted as an old healed infarct. Traces of amyloid were present within the media of the

fibrotic blood vessels in this area. None was seen elsewhere, neither in other parts of the lung nor in other organs. The liver weighed 1000 gm. The surface was slightly granular, and microscopic examination showed a distortion of architecture by a mild excess of fibrous tissue, radiating irregularly from the portal spaces. The nephrosclerosis, with kidneys weighing 105 and 115 gm., was characteristically arteriosclerotic in type. In the pancreas small areas of fibrosis affected the interstitial connective tissue, but the islands were intact.

MICROSCOPIC STUDY OF HEARTS

These 5 cases appear to form a single group and may be considered together. The ages of the patients ranged from 83 to 93 years. The causes of death varied greatly. The weights of the hearts ranged from 230 to 630 gm. The heart weighing 630 gm. showed severe Mönckeberg's sclerosis of the aortic valve, with an acquired bicuspid valve, and the hypertrophy seemed a direct result of this. Three of the hearts with weights of 420, 470, and 630 gm. showed considerable interstitial fibrosis but the other 2 (230 and 350 gm.) revealed no significant fibrosis within the myocardium. Arteriosclerotic changes, generalized, were prominent in all but case 1, but less so in case 4 than in cases 2, 3, and 5.

The microscopic examination of the hearts revealed fundamental similarity. In all there were small patchy masses of hyaline material, situated in the interstitial tissue, and surrounding muscle fibers singly and in groups. This hyaline material in all specimens reacted positively with Congo red and with methyl violet. The latter stain gave intense metachromatic reactions, while the Congo red reaction was less strong. The hyaline substance also stained positively with ammoniacal silver, according to the method given below. The general pattern of the amyloid, in all cases, is illustrated in Figures 1 and 2. The hyaline material (Fig. 1) at first glance suggests fibrosis. In foci where the involvement is intense, the muscle fibers have disappeared or are markedly shrunk and atrophic, as if choked by the surrounding collars of amyloid. Careful examination, however, shows absence of fibrillar texture characteristic of collagen, while the deposits are hyaline. Differential staining reactions are, of course, conclusive.

Where variable amounts of interstitial fibrosis were present, amyloid was observed not only in immediate contact with muscle fibers but also as occasional small plaques and masses of hyaline material within the fibrosed zones. These deposits of amyloid within areas of fibrosis were readily recognizable in preparations stained with hematoxylin and eosin, and were differentially shown by specific stains. Methyl violet

was most useful in this regard. The heart of case 3 revealed the most marked interstitial fibrosis in this series.

It must be emphasized, however, that in none of these cases was the total amount of amyloid very great, and certainly not comparable to the massive deposits reported occasionally in the literature. The hypertrophy in the largest heart in this series, 630 gm., was explained by the aortic valvular disease. In all 5 cases the amyloid appeared to be an incidental finding. There was not sufficient evidence to attribute death to the deposition of amyloid.

In cases 3, 4, and 5 small amounts of amyloid were noted in the lungs. In cases 3 and 4, chronic passive congestion of the lungs was present, and the small amyloid masses were situated in the thickened alveolar septa. In case 5 the hyaline substance was seen within arterial walls in a zone of old infarction. In none of the cases was amyloid observed in any other organ.

AMYLOIDOSIS WITH PYELONEPHRITIS

A sixth case of atypical amyloid disease appears to be in a totally different category, but may be reported at this time.

Case 6

The patient, 70 years of age, speaking practically no English, was admitted in a critically ill condition, with nausea, vomiting, and severe abdominal pain of about 1 week's duration. The clinical impression was acute intestinal obstruction. Laboratory data showed a white blood cell count of 22,700, with 95 per cent polymorphonuclear leukocytes, and 4 plus albumin in the urine, with many pus cells. He had had previous surgical treatment, as evidenced by old abdominal scars. Although his physical condition was poor, immediate laparotomy seemed indicated. Severe adhesions were encountered and freed, releasing kinked and adherent loops of small bowel. An enormously distended gallbladder was noted. This was drained, and the abdomen closed. In spite of supportive postoperative therapy, the patient died 20 hours after admission.

It was found, subsequently, that the patient had had a number of hospital admissions in various hospitals, over a period of at least 12 years. He had had a resection of the cecum and ascending colon, with ileocolostomy, for carcinoma of the colon, verified pathologically. Apparently, he had a partial resection of the stomach, with anterior gastrojejunostomy at another hospital, but this record has not been verified. In the 12-year period there were numerous hospital admissions for vomiting, pain in the upper abdomen, dizziness, abdominal discomfort, anorexia, fatigue, chest pain, and vague symptoms. There was some evidence that over the past 12 years numerous urine examinations showed albumin and white blood cells, but no particular significance had been attached to them.

The pathologic diagnoses following autopsy were: Nephrolithiasis, right; severe subacute and chronic pyelonephritis; chronic ureterocystitis; atypical amyloidosis, involving kidneys, and blood vessels of numerous organs; arteriosclerosis of coronary arteries; healed infarct of heart; chronic passive congestion of lungs; atelectasis; emphysema;

scars of old operations (partial colectomy with ileocolostomy for carcinoma of colon, gastrojejunostomy with partial gastric resection); recent cholecystostomy and separation of adhesions.

The right kidney, intact, weighed 250 gm. It contained a large stag-horn calculus, tightly surrounded by a rim of renal tissue. The dilated calyces contained abundant, greenish, purulent material. The persisting renal tissue, of greenish red color, showed severe reduction of the usual markings and of definition between cortex and medulla, and presented a somewhat mottled appearance. The right ureter had a diameter of 1.3 cm., with a dense, firm wall and a minute, slit-like lumen. The left kidney, weighing 220 gm., was granular, with a soft, bulging cut surface showing dulling but not obscuration of the renal markings. No hydronephrosis or calculi were present on the left and the left ureter appeared normal. The bladder was thin-walled, but with severe congestion of the mucosa. The prostate revealed slight hyperplasia. The heart weighed 370 gm., and showed an old healed infarct of the posterior left ventricular wall, extending into the septum. The coronary arteries exhibited extensive sclerosis and narrowing, but total occlusion was not discovered. Microscopic examination showed severe scarring and cellular infiltrations of the right kidney, with only slight fibrosis and pyelonephritis of the left. The right ureter and bladder revealed prominent chronic inflammatory changes and fibrosis. In both kidneys the glomeruli were involved by massive deposits of amyloid, of the usual subendothelial type (Fig. 3). In addition, the capsules of Bowman frequently were thickened by fibrous tissue, and many glomeruli were sclerosed and fibrotic without amyloid infiltrations. No amyloid was seen in relation to the tubules or interstitial connective tissue, but many of the small and medium-sized blood vessels contained lumpy hyaline masses in their walls. These masses stained positively for amyloid (Fig. 4). In the other organs amyloid was found in the blood vessel walls (similar to Figs. 4 and 5), in the heart, liver, gall-bladder, gastro-intestinal tract, adrenal capsule (Fig. 5), ureter, and bladder. None was observed in the parenchymatous tissues of these organs, but only in the walls of arteries and veins. None was seen in relation to capillaries or sinusoids. No amyloid could be identified in any part of the lung, spleen, or pancreas. In the heart the amyloid was observed only within the walls of the coronary vessels. None was observed surrounding the muscle fibers, as in cases 1 to 5.

In summary, this case, with very long-standing pyelonephritis and nephrolithiasis, showed amyloidosis limited to the renal glomeruli and to arteries and veins in many different organs.

CLASSIFICATION OF AMYLOID DISEASE

Without attempting an exhaustive review of the literature, certain facts stand out clearly. The most common manifestation of amyloid disease occurs in the course of tuberculosis. Perla and Gross,³ in a study of 1500 autopsies, reported 100 cases representing about 25 per cent of all patients dying of tuberculosis. In this form of the disease, the amyloid is most commonly distributed in the kidneys, liver, spleen, and adrenals; less frequently in one, two, or three of the above organs, but not in all.⁴ Huebschmann⁵ reported that in 8 of 9 consecutive autopsies on tuberculous patients with amyloidosis, the amyloid was found in the heart also. In addition, the amyloid may be found in traces in other organs, such as parts of the gastro-intestinal tract, pancreas, or salivary glands.⁶ Thus there is a certain pattern of distribution, which I wish to call *typical*, that is, predominantly in the parenchyma of kidneys, spleen, liver, and adrenals, less frequently and with less intensity in certain other sites. This typical form of distribution, called secondary in the literature, is seen in the course of tuberculosis, osteomyelitis, pyelonephritis, lung abscess, carcinoma of stomach,^{3,7} carcinoma of lungs, leukemia,³ tabes,³ Hodgkin's disease,^{7,8} multiple myeloma,⁹ rheumatoid arthritis,^{3,4,10} thermal burns,¹¹ and others.

The use of the term secondary for this type is entirely misleading. It implies that, somehow, the associated disease (tuberculosis, for example) is the cause of the amyloid. A philosophic discussion of causality is scarcely relevant to the present paper. One must distinguish, however, a proximal or immediate cause, which is both necessary and sufficient (for example, prolonged local anoxia as a cause of infarction); and a more remote link in the causal chain (for example, generalized arteriosclerosis as a "cause" of infarction) which is neither necessary nor sufficient to produce the given phenomenon, but which may initiate or induce the proximal cause.

In this sense it is obvious that the proximal cause of amyloidosis is not known, in spite of considerable experimental study and chemical analysis. However, the statistical correlation between tuberculosis and secondary amyloidosis is too high to be ignored. One must assume, therefore, that tuberculosis, while not the direct cause, is probably part of the causal chain, provided that certain other unknown factors also are present.

The rôle of chronic suppurative disease in the production of amyloidosis is probably similar to that of tuberculosis. However, the position of conditions such as arthritis or Hodgkin's disease is more

questionable. There simply is not sufficient evidence to implicate these diseases as part of the causal chain, although they cannot arbitrarily be excluded. Judgment must be suspended.

This necessity for suspending judgment is emphasized by the rare case of amyloidosis, with essentially typical distribution, in which no other disease is observed. In various published cases,¹²⁻¹⁰ for example, no associated disease was present, but the distribution of amyloid was approximately comparable to that seen in the so-called secondary type. Since we are ignorant of the true cause of amyloidosis, to call one group secondary and the other primary seems illogical. One might suggest that the true proximal (but unknown) cause was the same in both groups. In the alleged secondary group an associated disease might or might not be a remote factor of the causal chain; in the so-called primary group the causal chain is unknown throughout. In some instances, *e.g.*, case 2 of Dillon and Evans,¹⁵ an associated disease (bacterial endocarditis) was present, but the authors nevertheless considered the case primary. In our state of ignorance such nomenclature is utterly confusing.

There is a second major category of amyloid disease which differs from the preceding in the sites of deposit of the amyloid. This category includes most of the so-called primary or atypical cases. Lubarsch,⁶ and others, have discussed this group. The important criteria are: (1) Deposition of amyloid in unusual sites (heart, lungs, striated and smooth muscle, skin); (2) sparing of the usual or typical sites (spleen, kidneys); (3) absence of demonstrable cause. Inconstancy of staining reactions may or may not be present. The literature of these cases has been most admirably reviewed by Lindsay,² and repetition is unnecessary.

Since the cause of amyloid deposition is not known, classification by etiology is not possible; and since there is no constancy in the clinical picture, clinical considerations must be kept in the background. The simplest classification, taking into account the known factors, and not stressing hypothetic factors, would be on the basis of anatomic distribution. The following is suggested.

1. *Typical amyloidosis*: Deposition of amyloid in the usual sites (kidneys, spleen, liver, adrenal, etc.)
 - a. Associated with other disease (as, tuberculosis, multiple myeloma, carcinoma, osteomyelitis)
 - b. Not associated with other disease (rare, but occasionally reported as "primary")
2. *Atypical amyloidosis*: Amyloid, not following the usual or typical

distribution, found in one or many foci or organs, with or without symptoms

- a. Associated with other disease or conditions (as, multiple myeloma, Hodgkin's disease, carcinoma, pyelonephritis, bronchiectasis, and the like)
- b. Not associated with other disease (including most of the cases reported in the literature as primary amyloidosis, whether systemic or local)

For purposes of classification it is necessary to be somewhat arbitrary, and it is proposed that the parenchymatous involvement of liver, spleen, kidneys, and adrenals be considered *typical*. The addition of other organs (for example, the unusual cases of Gerber¹⁷ and Edens¹⁸) should not be sufficient to remove it from this category. On the other hand, *atypical* should be applied to those cases in which liver, spleen, kidneys, and adrenals are spared, or in which only one of them is involved with extensive amyloid in less usual sites as defined by Lubarsch.⁶ This is admittedly an arbitrary division, but the diversity of cases is so great that otherwise no simple schema is feasible.

The most widely accepted classification hitherto is that of Reimann, Koucky, and Eklund,¹⁹ who defined four groups: Primary, secondary, amyloid with multiple myeloma, and tumor-forming amyloid. More logical is the classification of Rosenblum and Kirshbaum,²⁰ who divided amyloidosis into primary or idiopathic and secondary or symptomatic, with subdivisions in each group of diffuse or typical, and localized or atypical.

It is the contention of this paper that there is no warrant for the use of the terms primary and secondary. Lindsay² has already expressed objection to these terms, suggesting "that when the basic mechanism is known, primary amyloidosis will be classified as a 'secondary' type."

Amyloidosis, however, takes so many different forms that a few words of comment are in order. One of the most interesting groups is that associated with multiple myeloma. Of approximately 650 cases reported in the literature,^{9, 21, 22} concomitant amyloidosis was noted in 41. Most often there is an atypical distribution, but sometimes, as far as can be determined from available data, the distribution is comparable to that seen after tuberculosis, designated typical in this paper. Reimann et al.¹⁹ would call this group neither primary nor secondary, but would relegate it to a separate category. Similarly, so-called tumor-forming amyloidosis which may involve bones,^{23, 24} upper respiratory tract,²⁵ or conjunctiva²⁶ need not have a separate niche apart from

other forms, but can be grouped with atypical amyloidosis. Some of these cases are associated with multiple myeloma,^{21, 22} others show no associated disease. The classification proposed herein readily accommodates these cases. The skin disease lichen amyloidosis,²⁷⁻³⁰ frequently reported in the dermatologic literature, is another example of atypical amyloidosis. There seems no need for separate categories or divisions for each of the many examples of localization of amyloid.

ATYPICAL AMYLOIDOSIS, ASSOCIATED WITH SENILITY

The first 5 cases reported in this paper appear to form a single group, all showing amyloid in the heart, with little or no localization elsewhere. The amount of amyloid was relatively small compared to that in some of the cases described in the literature,² and was found incidentally at autopsy. All of the patients were 80 years of age or over. They all showed other lesions adequate to explain death, and the only common feature was advanced age. These cases apparently are identical with the 3 reported by Ranström,³¹ in patients 80, 81, and 88 years of age. The older literature contains a brief note by Beneke³² reporting 6 similar cases in old individuals, but the ages are not given. The case of Beneke and Bönning³³ is probably in the same category, but again age and clinical data are not presented.

The conclusion seems justified, on the basis of my own and other cases, that the amyloid deposition in the heart without clinical symptoms, can be correlated with old age. It appears to be entirely misleading to consider such cases as primary. It is of interest that of the 5 cases, 4 were found in a period of 16 months, in a total of 193 autopsies. In this small series 19 patients were 80 years of age or over, giving a percentage of 21 for that age group. This suggests that the condition is far more prevalent than has hitherto been suspected.

There is independent evidence that under certain circumstances amyloid deposition is a function of age. In an investigation of amyloid in the genito-urinary tract, Bursell³⁴ studied deposition in the seminal vesicles and found an incidence increasing with age. Thus, in the age group 76 to 90, of 38 cases, amyloid was found in 13. Its deposition was not related to inflammation in the prostate. It is plausible that his cases belong in the same category of atypical amyloidosis associated with old age. In my own material, unfortunately, the seminal vesicles were not examined microscopically, but it is only a question of time until the correlation between the two groups is proved or disproved.

Why the advanced age group should show a significant amount of amyloid is not clear. It is possible, as suggested by Warren,³⁵ that atypical

amyloidosis represents a "widespread perversion of function" of the connective tissue, with dysfunction of fibroblasts. Possibly local ageing of fibroblasts may be a relevant factor. In this connection it is of interest to note the occasional finding of amyloid in sclerosed pancreatic islands of many diabetic subjects (atypical amyloidosis associated with diabetes), an observation indicating the importance of local factors.

ATYPICAL AMYLOIDOSIS ASSOCIATED WITH PYELONEPHRITIS

In the sixth case reported here, with a massive nephrolithiasis and severe pyelonephritis, the amyloid showed a curious localization. Its parenchymatous distribution was limited to the renal glomeruli, but in addition it was found in the walls of small blood vessels in many organs, including the heart. This distribution is unusual, and deserves the designation of atypical. We may assume that the concomitant pyelonephritis was the most significant associated disease. It is reasonable to designate this case as atypical amyloidosis associated with pyelonephritis. It is plausible that the infection, in this instance, had the same causal importance that tuberculosis or osteomyelitis has in typical amyloidosis. The peculiar localization is not explained. (The case suggests some similarities to that of Binford.³⁶)

Three important unsolved problems haunt the subject of amyloidosis: (1) Why does amyloid disease usually take a typical distribution, but occasionally an atypical localization? (2) What is the true proximal cause (or causes) of amyloid production (as contrasted with inciting or mediating factors or associations, such as the familiar tuberculosis)? (3) What, if any, are the essential chemical differences between the typical and atypical amyloid? It is probable that a thorough-going answer to any of these problems will automatically solve all of them.

STAINING OF AMYLOID WITH SILVER

Variations in the staining properties of amyloid have long been noted. The atypical form, especially, shows much inconstancy in respect to the usual tinctorial reactions. Hass and Schulz³⁷ have demonstrated the chemical complexity of the usual or atypical amyloid. Similar work has not been done with the atypical forms. Adequate chemical analysis may well display many differences. Meanwhile the tinctorial, or crudely histochemical studies must be relied on.

That amyloid can be impregnated with silver first came to my attention while studying with the late Dr. Pio del Río-Hortega. In the course of impregnating reticulin fibers of the spleen, some amyloid which was pres-

ent stained beautifully, in a tone different from either collagen or reticulin. This impregnation aroused no interest or special comment, and was accepted in his laboratory as a well known phenomenon. In the course of subsequent studies on silver impregnations of the nervous system, a simple method was adapted for the differential exhibition of senile plaques and Alzheimer strands.^{38,39} This method, based on one of Hortega's nuclear stains, was found to impregnate amyloid with facility and clarity. A brief resumé of the method is given.

Thin frozen sections are washed in water, and placed in an ammoniacal silver solution. To 5 cc. of 10 per cent silver nitrate, there is added, dropwise, enough ammonia to produce and then just dissolve the characteristic precipitate. Then 6 to 8 cc. of sodium carbonate solution are added. Of crystalline sodium carbonate, I used a 5 per cent strength; of the anhydrous form, 3.5 per cent. Or, saturated lithium carbonate may be used as the added alkali. The resulting solution is diluted to 75 cc. with distilled water. To about 10 cc. of this solution, a few drops of pyridine are added and the solution gently heated in a small, covered beaker and lightly agitated until the sections turn a tobacco-brown. The temperature should not exceed about 45° C., which can be tested by applying the bottom of the beaker to the back of the hand. The brown sections are then washed in sodium thio-sulfate ("hypo"), followed by water, and are mounted without toning. *No formaldehyde or other reducing agent is employed at any point.* The method gives indifferent results in paraffin sections, which can be kept in the silver solution in the paraffin oven until they turn a rich brown. This varies from ½ to 2 hours. The staining is far more diffuse than with frozen sections, and muscle cytoplasm is too deeply impregnated. Paraffin sections are not recommended.

Examples of frozen section impregnation with this method are shown in Figures 2, 3, 4, and 6. The amyloid stains deeply, but nuclei and lipochrome pigment also are deeply impregnated. Cytoplasm is frequently stained a light brown, and a few wisps of collagen may impregnate. Sometimes protein casts in the lumina of renal tubules stain with moderate intensity. Good silver impregnations were obtained in all 6 cases reported here. The results are permanent. Preparations from case 1 are vivid after a lapse of 6 years. In Figure 6 is shown an amyloid liver from a case of tuberculosis, to illustrate the staining of so-called secondary amyloid.

The method is more cumbersome than the methyl violet or Congo red reaction, and is not suggested as a substitute. It is presented as showing an interesting chemical property of amyloid, namely, an intense argyrophilia.

The reaction of amyloid with ammoniacal silver offers a new tool. The method is reported, not as a substitute for the simpler staining technics, but as a hitherto undescribed property of amyloid. It was previously shown in studies on senile brains³⁹ that the intracellular, Alzheimer strands as well as the interstitial senile plaques were strongly argyrophilic. That is, ammoniacal silver, facilitated by gentle heat, would impregnate these structures with regularity and intensity without

the use of any reducing agent. Nuclear material and lipochrome pigments also were regularly impregnated by the same method. Nerve fibrils and some connective tissue fibrils might be inconstantly and lightly stained. In the studies on amyloid the staining is not entirely selective, for, as can be seen in the illustrations, nuclei and lipochrome stain deeply. In general there is very good differentiation between amyloid and collagen, but occasionally some strands of heavy collagen will darken markedly with this technic. Further work is needed to elucidate the relationships and to correlate argyrophilia with other tinctorial properties.

SUMMARY

The present-day classification of amyloid disease, with its distinction of primary and secondary, involves the user in inevitable inconsistencies. A new classification is proposed, based on typical or atypical distribution, and on association or lack of association with other diseases or conditions. With this classification, a new category, "atypical amyloidosis associated with senility," is presented. Five such cases are described, in subjects over 80 years of age in whom the amyloid was present almost exclusively in the heart, and only as an incidental autopsy finding. A further case of a different category, atypical amyloidosis associated with pyelonephritis, also is presented.

A newly described chemical property of amyloid is its ability to combine with ammoniacal silver without the use of any reducing agent. A simple technic is described for the application of this method, which proves to be a useful tool for the discovery and identification of this substance.

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[Illustrations follow]

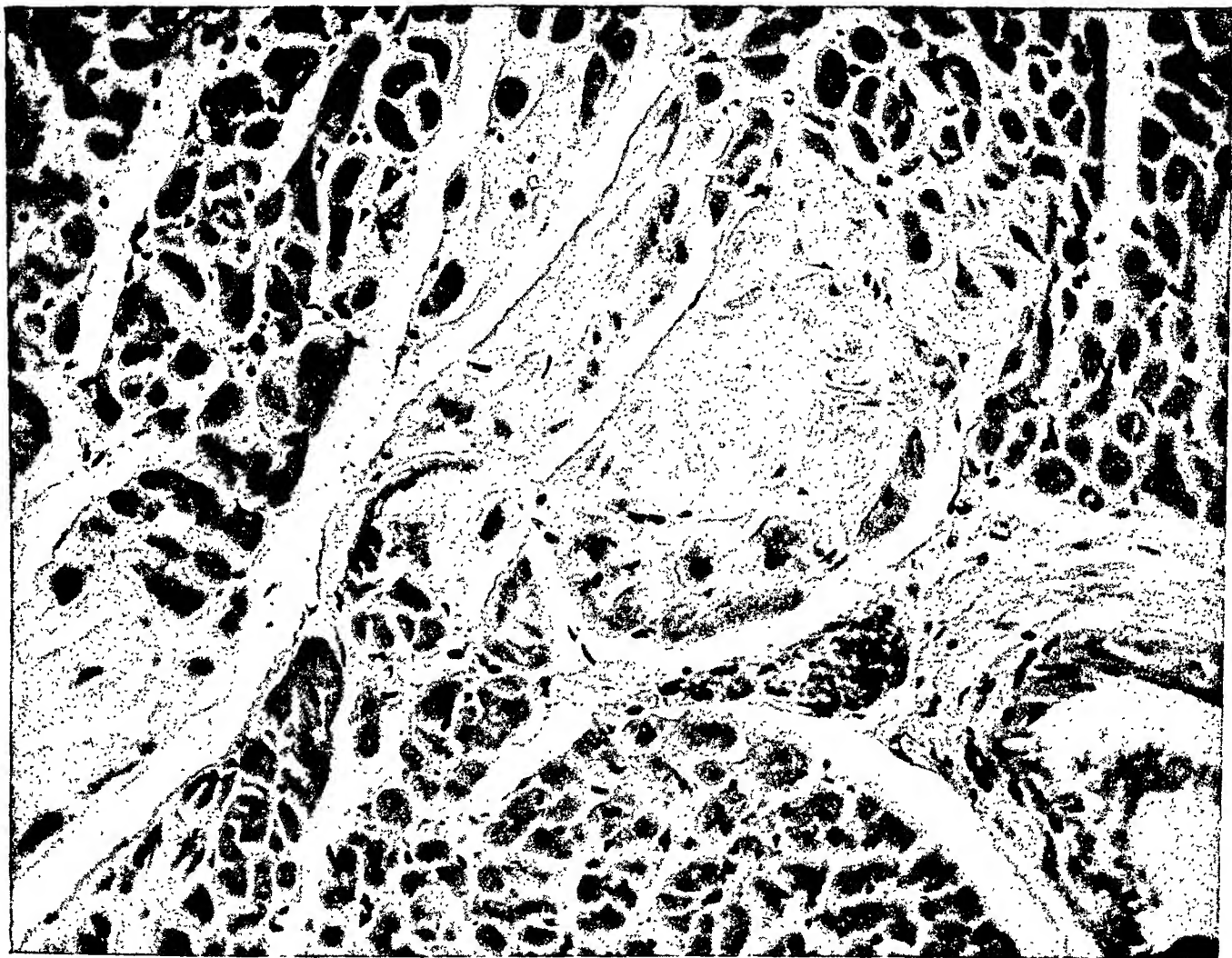
DESCRIPTION OF PLATES

PLATE 178

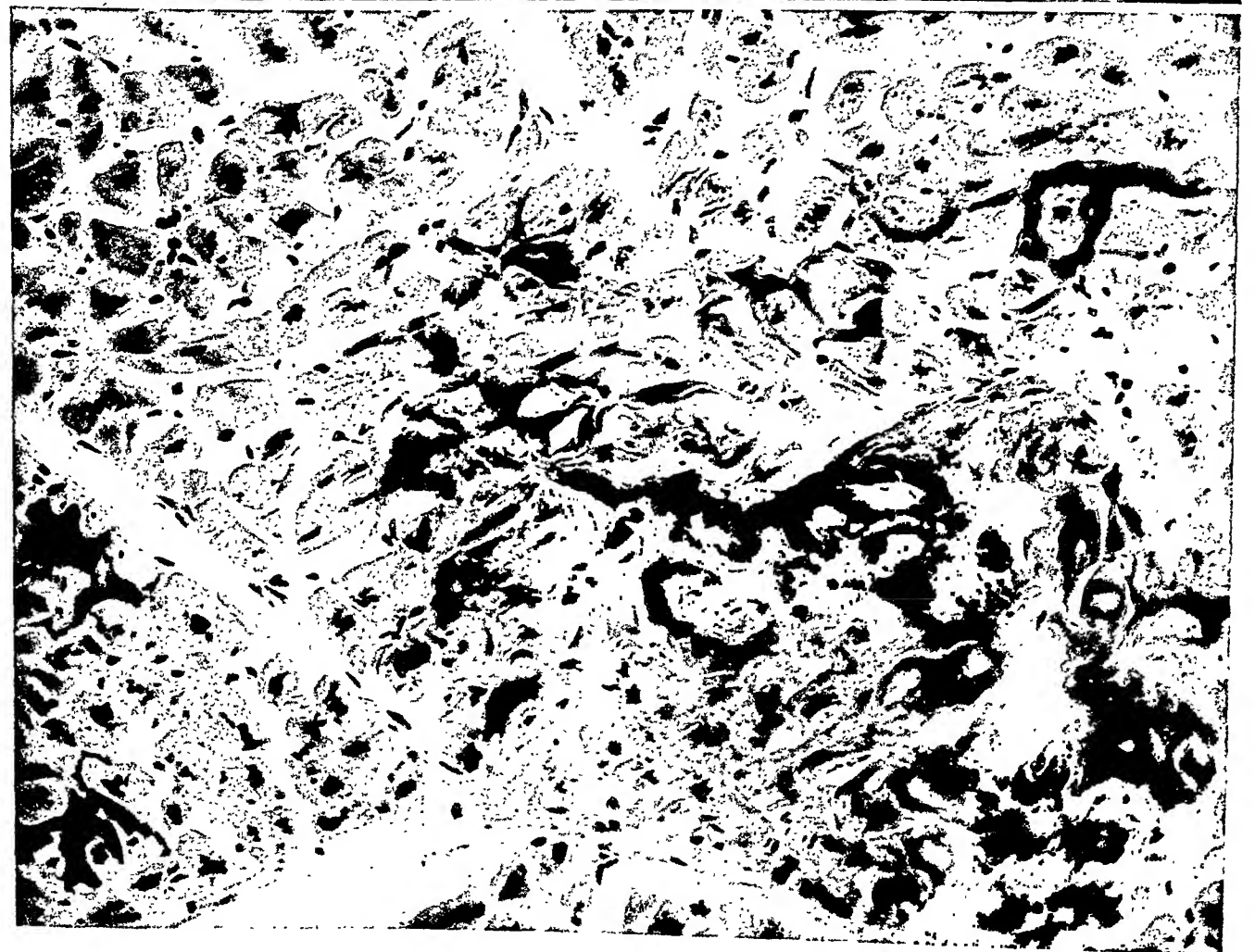
FIG. 1. Myocardium, case 4. Patches of amyloid occupy the interstitial tissue, replacing many muscle fibers and surrounding others in ring-like fashion. Many of the surrounded fibers are atrophic. Hematoxylin and eosin stain, paraffin section. $\times 600$.

FIG. 2. Myocardium, case 4. The amyloid appears as dark-staining, lumpy masses surrounding some individual muscle fibers and replacing others. The interstitial connective tissue is essentially unstained, but nuclei and lipochrome pigment are impregnated. Silver impregnation, frozen section. $\times 600$.

1



2



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PLATE 179

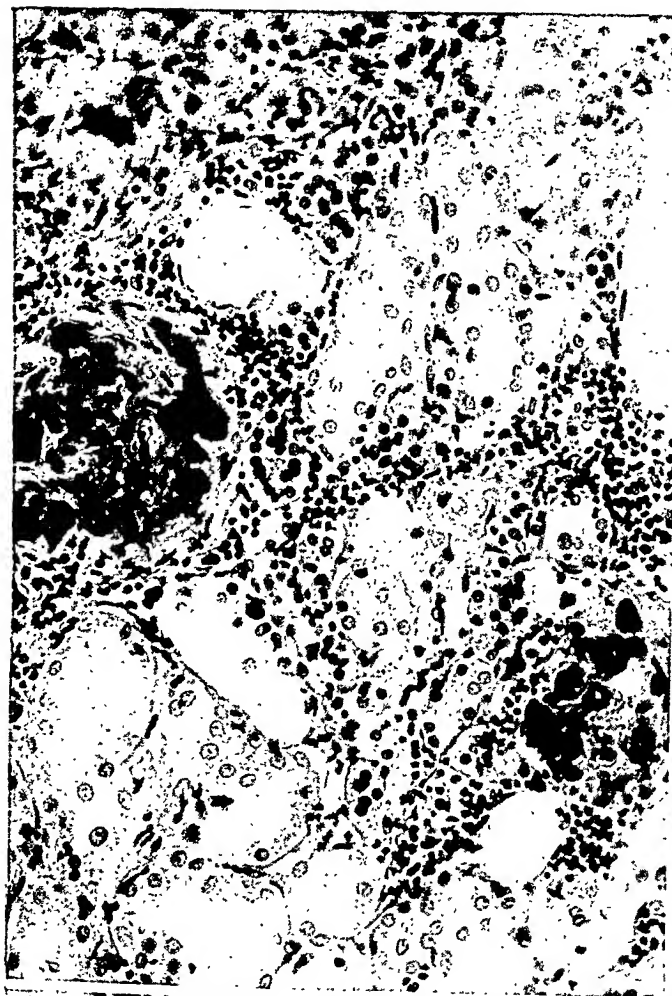
FIG. 3. Kidney, case 6. Amyloid within the glomeruli is heavily stained, and contrasts well with the connective tissue of the thickened Bowman's capsules. Nuclei are well shown. Silver impregnation, frozen section. $\times 500$.

FIG. 4. Kidney, case 6. Amyloid is seen within the walls of a small blood vessel. Silver impregnation, frozen section. $\times 500$.

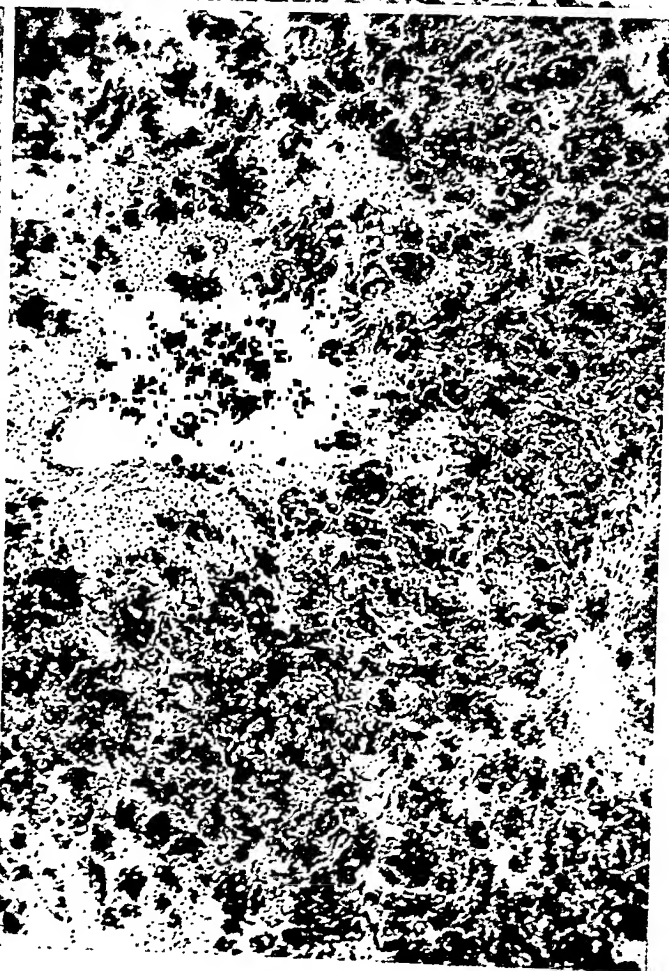
FIG. 5. Adrenal, case 6. The amyloid in one of the periadrenal blood vessels is well shown. The general appearance after Congo red staining is similar to that seen with silver impregnation. No amyloid was present in the adrenal gland proper. Congo red and hematoxylin, paraffin section. $\times 500$.

FIG. 6. Amyloid liver from a case of tuberculosis. In the material studied to date, there is no difference in reaction to silver between typical and atypical amyloidosis. At this magnification nuclei are visible as small dots. The extensive amyloid distribution is well shown. Silver impregnation, frozen section. $\times 55$.

3



5



King

OBSERVATIONS IN GUINEA-PIGS FOLLOWING INJECTION OF SPECIFIC HEMATOPOIETIC SUBSTANCES DERIVED FROM URINES OF HUMAN LEUKEMIC SUBJECTS *

ARTHUR SAWITSKY, M.D.,† and LEO M. MEYER, M.D.

(From the Department of Therapeutics, New York University,
College of Medicine, New York, N.Y.)

In a previous report,¹ the effects of the injection of extracts of beef liver into guinea-pigs were described. The theoretic considerations that prompted the investigation and a brief summary of the literature were given. This report is concerned with a description and evaluation of the observations in guinea-pigs of the effects of extracts of urines of leukemic subjects.

MATERIALS AND METHODS

Thirty-three young male guinea-pigs were used. Weights ranged from 205 to 520 gm. Two animals, however, weighed 700 and 780 gm., respectively. These animals were standardized and observations made as described in the preceding paper.¹

Urinary extracts were prepared in the following manner. Urines of patients with proved myeloid or lymphoid leukemia were collected in bottles containing chloroform and pooled according to type. The urine was adjusted with hydrochloric acid to pH 1 to 2, heated to boiling for 5 to 10 minutes, and then extracted with chloroform. The chloroform extract was concentrated, saponified in 2N sodium hydroxide solution, and extracted with ether to remove the neutral materials. The alkaline solution was saturated with carbon dioxide and extracted with ether to remove phenolic materials, then acidified with hydrochloric acid and re-extracted with ether. The ether extract was evaporated to dryness and extracted with petroleum ether. The petroleum ether extract was extracted with methanol to remove benzoic materials and made into lead salts in a hot alcoholic solution, which was then filtered. The alcohol-insoluble lead salts were extracted with ether to remove ether-soluble lead salts. The ether-insoluble lead salts were regenerated to acid form, dissolved in acetone, and kept at -20°C . for crystallization. They were then filtered to remove crystals of palmitic and/or stearic acids. The filtrate was concentrated and separated by succination into carbinols and noncarbinols. Extracts were then suspended in normal butyl succinate so that 1 cc. of extract suspension was equivalent to 1 l. of the original urine. One chloroform-extracted carbinol sample

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† Lederle Fellow in Hematology.

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was suspended in cottonseed oil and 1 cc. of that suspension was equivalent to 1.33 l. of the original urine.

A second type of extraction, producing "ether extracts," was prepared in the following manner. Urines were pooled as described above, 2 per cent potassium hydroxide added, and the urine was then boiled and refluxed with steam under nitrogen for 4 hours. Upon cooling, neutral substances were removed with three chloroform extractions. The alkaline solution was acidified with sulfuric acid and three extractions with ether were made to remove the acidic substances. The extract was washed with water until neutral and then concentrated by steam heat under vacuum. The extract was suspended in normal butyl succinate so that 1 cc. of extract was equivalent to 1 l. of the original urine.

In order to insure potency of the extracts tested, the carbinol fractions used were those made from urines of patients with lymphoid leukemia. Similarly, the noncarbinol fractions used were those derived from the urines of patients with myeloid leukemia.

DESCRIPTION OF PATHOLOGIC CHANGES

The gross and histopathologic changes observed were essentially the same as those noted in the preceding paper.¹

OBSERVATIONS

In general, the local reactions to the urinary extracts were more severe than those observed with liver extracts. The animals appeared more ill. Ruffled fur, lethargy, and somewhat stertorous breathing were common. Ulceration of the hind feet occurred after the larger doses. The peripheral blood picture was not significantly altered except in occasional animals in which anemia was marked. In such, normoblasts and occasional myelocytes were noted in the smear. Weight loss during the course of the experiment was usual. In 21 animals spontaneous death terminated the experiment.

Carbinol (Lymphoid) Chloroform Fraction from Urines of Patients with Lymphoid Leukemia

Two different samples of chloroform extract were used. One sample was suspended in cottonseed oil. Normal butyl succinate was the medium used for the second fraction (Table I.)

The cottonseed suspension in doses of 0.2 cc. was injected, daily, intramuscularly into alternate hind limbs of 3 young male guinea-pigs weighing 239, 250, and 263 gm., respectively. Each animal received a total dose of 1.8 cc., representing 6.0 l. of the original urine. Periph-

eral blood findings remained normal. The curve of gain in weight was flattened slightly. After 0.8 cc. of extract, ulceration of the plantar surface of the right hind foot was noted in one animal. All animals showed some ruffling of hair, but appeared well otherwise. The experiment was terminated 7 and 9 days after the final injection. Two guinea-pigs were observed to have lymphoid responses, the other animal was negative.

Six male guinea-pigs weighing 240 to 300 gm. and one male weighing 700 gm. were given daily intramuscular or subcutaneous injections of

TABLE I
*Results of Injection of the Carbinol (Lymphoid) Chloroform Fraction from
Urine of Patients with Lymphoid Leukemia*

Guinea-pig no.	Initial weight	Extract, total dose	Equivalent urine	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	cc.	l.	days	days		millions	millions	thousands	thousands	
74	700	10.25	10.25	20	0	D	5.35	5.8	16.0	12.7	+ L
81	263	4.5	4.5	12	1	D	5.2	5.0	22.7	19.0	None
83	240	0.5	0.5	2	0	D		4.98		13.0	None
84	285	6.0	6.0	15	1	D	5.15	4.4	11.5	13.5	+++ L
86	255	5.25	5.25	13	0	D	5.3	4.8	12.3	15.0	+++ L
87	300	1.25	1.25	6	1	D	5.1	5.3	12.6	12.6	+ L
88	280	5.5	5.50	13	1	D	5.2	5.4	14.6	30.0	+ L

Key: L = lymphoid reaction; D = died.

the butyl succinate suspended fraction. Initial dosage was 0.25 cc. This was increased every 5th day by 0.25 cc. until each animal was receiving 1.0 cc. daily. Injections were continued until death. Total dosage varied from 0.5 to 6.0 cc. except for the largest animal which received 10.25 cc. After 2 weeks of injections and a total dose of 5.25 cc., the left hind foot of one animal showed gangrenous changes which progressed until death 2 days later. The 8th day after the initial injection, all animals had ruffled fur and appeared to be ill. The hind limbs (sites of injection) became indurated. All animals lost weight during the experimental period. Peripheral blood changes were not significant. The animals survived from 2 to 20 days after the initial injection. Five died within 15 days, the 6th on the 20th day. Four animals had enlarged cervical nodes. The thymus was prominent in all. Two presented enlarged mesenteric nodes and in one the right inguinal node was enlarged. In the 4 animals with enlarged lymph nodes, lymphoid hyperplasia and infiltration of the cervical nodes (Fig. 1), and to a lesser extent of the liver (Fig. 2), adrenal, kidney, and lung (Fig. 3), were noted. The spleen was involved in only one animal (Fig. 4).

The bone marrow could not be studied adequately because of technical difficulties. The remaining 3 were negative except for some round-celled infiltration of the medulla of the adrenals and of the kidneys. The 4 positive animals received 5.25 cc. or more of extract.

Carbinol (Lymphoid) Ether Fraction from Urines of Patients with Lymphoid Leukemia

Three young male guinea-pigs weighing 275 to 430 gm. were given daily injections of 0.5 cc. of carbinol ether extract intramuscularly in alternate thighs. The total doses were 1.5, 2.0, and 4.5 cc. Death occurred 4, 5, and 13 days after the initial injections. The animal receiving the largest dose showed enlargement of the cervical lymph nodes and lymphocytic hyperplasia of the cervical, axillary, and mesenteric lymph nodes. The spleen was hyperplastic and infiltration of the bone marrow was observed (Fig. 6). Another animal showed lesser but similar changes in the spleen, adrenals, and lung, while the third revealed round-celled infiltration of the bone marrow but myeloid changes in the cervical nodes, spleen, adrenals, and lung.

Two animals were given a different sample of similarly prepared extract in the manner described above. A total dose of 4.0 cc., equivalent to 4.0 l. of urine, was injected. The animals appeared ill after the fourth injection and one developed bilateral gangrene of the hind limbs. They were sacrificed 8 and 20 days after the last injection. The bone marrow of both animals revealed round-celled infiltration, but the cervical nodes, spleen, liver, adrenals, and lung showed mild to moderate degrees of myeloid infiltration and metaplasia.

Noncarbinol (Myeloid) Chloroform Fraction from Urines of Patients with Myeloid Leukemia

Six male guinea-pigs ranging in weight from 382 to 780 gm. were injected subcutaneously with daily doses of 0.25 cc. of the noncarbinol chloroform fraction (Table II). Every 5th day the dose was increased by 0.25 cc. until 1.0 cc. was given daily. Subcutaneous injection caused indurated and partially ulcerated areas in the skin, and the intramuscular route was used when this occurred. After 9 days of injections, all 4 smaller animals had gangrenous and ulcerative changes of the toes of the hind feet. The animals were clinically ill. There was progressive deterioration, and loss of weight was noted in all. Severe anemia in one animal and mild anemia in 2 others was observed at this time. There was concomitant leukocytosis in all 3. In the guinea-pig showing the marked anemia, normoblasts and myelocytes were seen in the periph-

eral blood. There was one anaphylactoid death during the course of the experiment. Five animals died and one was sacrificed 20 to 35 days after the initial injection. The total dosage varied from 8.75 to 11.75 cc. of extract, representing 8.75 to 11.75 l. of urine. Peritoneal irritation was observed in 2 animals. The cervical nodes were not enlarged. The thymus was difficult to identify. In 5 animals the liver was enlarged but of normal color. Four spleens were strikingly enlarged and in these animals the adrenals were likewise enlarged. Testicular atrophy was noted in 5 instances. Marked necrosis and suppuration were present in all cases at the various sites of injection. Microscopically, myeloid metaplasia and marked infiltration of imma-

TABLE II

Results of Injection of the Noncarbinol (Myeloid) Chloroform Fraction from Urines of Patients with Myeloid Leukemia

Guinea-pig no.	Initial weight	Extract, total dose	Equivalent urine	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
				Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	cc.	l.	days	days		millions	millions	thousands	thousands	
75	780	11.75	11.75	24	0	D	5.2	5.8	9.4	14.0	++++ M
76	700	11.75	11.75	35	11	S	5.45	6.5	5.6	25.0	++++ M
77	472	8.75	8.75	20	0	D	4.9	4.3	9.3	19.5	++++ M
78	465	11.75	11.75	25	1	D	5.5	2.97	8.3	29.6	+++ M
79	425	9.75	9.75	22	1	D	5.01	4.3	8.6	18.6	+++ M
80	382	11.75	11.75	26	2	D	5.6	4.3	9.0	12.6	++ M

Key: M = myeloid reaction; S = sacrificed; D = died.

ture myeloid elements in the lymph nodes, spleen, liver (Figs. 7 and 8), adrenals, and lung (Fig. 9) were observed in 5 of the 6 guinea-pigs.

Hyperplasia of myeloid elements of the bone marrow was present in all (Fig. 10).

Noncarbinol (Myeloid) Ether Fraction from Urines of Patients with Myeloid Leukemia

There were two samples of noncarbinol ether extract identically prepared, but injected at different times. Each cc. of extract represented 1 l. of urine. Two guinea-pigs were used for each assay. All 4 animals were males and varied in weight from 400 to 520 gm. Daily intramuscular injections of 0.5 cc. were given.

In the first assay, one animal received 3.5 cc. and the other, 4.0 cc. Both lost weight and were clinically ill. One became anemic, but no immature cells were noted in the peripheral blood of either guinea-pig. One died 10 days after the first injection, the other was sacrificed 12 days later. Peritoneal reaction to the extract was noted in both. The

cervical nodes of one animal were enlarged. Induration and necrosis of muscle at the sites of injection were moderate. Microscopic examination disclosed myeloid metaplasia and myeloid infiltration of the spleen, liver, adrenals, and kidneys, with hyperplasia of the bone marrow. The cervical nodes of one guinea-pig were similarly involved.

In the second assay, 2 guinea-pigs received a total dose of 4.0 cc. They remained clinically well until sacrificed 10 and 22 days after the final injection. The peripheral blood was unchanged. Gross examination revealed only mild induration at the sites of injection. Microscopic examination was negative except for a mild myeloid response in the bone marrow of both animals and in the spleen of one.

Normal Butyl Succinate Control

Three male guinea-pigs weighing 260 to 368 gm. were chosen as controls (Table III). They were given daily alternate subcutaneous

TABLE III
Normal Butyl Succinate Control

Guinea-pig no.	Initial weight	Extract, total dose	Elapsed time after		Death	Red blood cells		White blood cells		Reaction
			Initial injection	Last injection		Initial	Terminal	Initial	Terminal	
	gm.	cc.	days	days		millions	millions	thousands	thousands	
89	320	12.00	33	11	S	5.2	5.10	10.5	9.25	+ L
90	368	12.00	33	11	S	5.35	5.05	8.8	10.5	+++ L
93	260	12.00	23	1	D	5.05	4.95	11.0	14.0	± L

Key: L = lymphoid reaction; S = sacrificed; D = died.

and intramuscular injections. Initially, 0.25 cc. was given and the dose was increased by a like amount every 5th day until the daily dose was 1.0 cc. All 3 animals received a total dose of 12.0 cc. The first 6.0 cc. was well tolerated without abnormal clinical or hematologic signs. With larger doses, ruffled fur and breakdown of the sites of injection were noted. In one animal, gangrene of the left foot was observed. Weight was maintained during the injection period and then weight gains were noted. One animal lost weight after 10.0 cc. was given and died 3 days later. The other animals were sacrificed 11 days after the final injection. No side reactions were observed during the course of the experiment. Gross pathologic changes were limited to mildly enlarged cervical nodes in all animals and moderate induration and necrosis of the tissue at the sites of injection. Microscopic examination revealed mild to moderate lymphoid stimulation of the lymph nodes and spleen. The lungs of 2 animals were moderately involved. The bone marrows of all 3 guinea-pigs were negative.

DISCUSSION

We do not believe that we have produced leukemia, as we recognize the spontaneous form in animals and man. We have been able to produce leukemia-like lesions of either the lymphoid or myeloid type by the injection into guinea-pigs of substances of the carbinol or non-carbinol type, respectively. The fractions used are obviously crude. To what extent the menstruum is influencing the final result is difficult to state, but it is noteworthy that animals receiving carbinol (lymphoid) extract suspended in cottonseed oil manifest lymphoid stimulation. Animals receiving cottonseed oil alone tend to show myeloid stimula-

TABLE IV

Frequency of Organ Involvement Following Use of the Carbinol (Lymphoid) Chloroform Extracts from Urines of Patients with Lymphoid Leukemia

	Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
Suspended in cottonseed oil	± L	1		3	2			3
	L	1	1			1	1	
	None		2		1	2	2	
Suspended in normal butyl succinate	± L	1		4	2	4	1	
	L	4	1	1			2	
	None	2	6	2	5	3	4	

Key: L = lymphoid reaction.

tion if any response is elicited. Conversely, the noncarbinol (myeloid) fraction suspended in normal butyl succinate may show the most marked myeloid metaplasia and infiltrative response, while the succinate alone is lymphoid stimulating. In general, our results confirm those of Miller and his group² and the report of Heinle and his co-workers.³

Using both beef liver and urinary extracts, it was found that the noncarbinol fractions produce the more striking lesions. The separation of extract into carbinol and noncarbinol fractions is possible, but in our hands three times the equivalent dose of urine (in liters) has been necessary to produce lesions comparable with those described by Miller and his group.² We have found the fractions obtained by crude chloroform extraction to be more potent than the ether-extracted or the water-soluble forms.

The frequency of organ involvement is shown in Tables IV, V, VI, and VII. The lesions of the carbinol (lymphoid) fractions were most frequent in the lymph nodes, liver, lung, kidney, and bone mar-

TABLE V

Frequency of Organ Involvement Following Use of the Carbinol (Lymphoid) Ether Extracts from Urines of Patients with Lymphoid Leukemia and Suspended in Normal Butyl Succinate. (The Results from Two Extracts, Prepared Identically, Are Shown in the Upper and Lower Portions of the Table.)

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
± L				I	I		3
L	I	2				I	
± M	I				I		
M		I				I	
None	I		3	2	I	I	
L							2
± M	2		I	I	I		
M		2	I		I	I	
None				I		I	

Key: L = lymphoid reaction; M = myeloid.

row. The animals stimulated by the noncarbinol (myeloid) fractions showed lesions most frequently in the spleen, bone marrow, liver, lymph nodes, and adrenal cortex. The tables indicate pulmonary involvement in the myeloid reaction. In the majority of sections this took the form of a decrease or absence of normally present lymphoid tissue. Another point of interest not shown is the amount of thymic tissue present. We had no difficulty in identifying and removing the thymus of the carbinol (lymphoid) animals; it was often extremely difficult to do so in the noncarbinol (myeloid) group.

It was quite evident throughout the entire experiment that stimulation of myeloid tissue caused a reduction of lymphoid tissue in organs where lymphoid tissue was normally present and abundant, and vice

TABLE VI

Frequency of Organ Involvement Following Use of the Noncarbinol (Myeloid) Chloroform Extract from Urines of Patients with Myeloid Leukemia, Suspended in Normal Butyl Succinate

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
± M				I	I	2	3
M	5	5	5	2	4	3	3
None	I	I	I	3	I	I	

Key: M = myeloid reaction.

versa. The peripheral blood, however, did not share in this relationship. In only one animal (guinea-pig 78, Table II) were immature myeloid cells, myelocytes, and nonsegmented neutrophils found in the peripheral smear. This was true in spite of evidence of myeloid stimulation in all bone marrows of guinea-pigs given noncarbinol (myeloid) fractions of urinary extract.

In the previous report¹ we commented on the cellular infiltrations observed at the sites of injections. It was again observed that the non-carbinol (myeloid) fraction of urinary extract produced marked granu-

TABLE VII

Frequency of Organ Involvement Following Use of the Noncarbinol (Myeloid) Ether Extracts from the Urines of Patients with Myeloid Leukemia and Suspended in Normal Butyl Succinate. (The Upper and Lower Portions of the Table Show the Results from Two Extracts Identically Prepared.)

Reactions	Lymph nodes	Spleen	Liver	Kidney	Adrenal	Lung	Bone marrow
± M	1		1	2	1	1	1
M		2	1		1		1
None	1					1	
± M		1					2
M							
None	2	1	2	2	2	2	

Key: M = myeloid reaction.

locytic reaction with occasional areas of suppuration. In the carbinol (lymphoid) stimulated animals, few inflammatory cells were seen. The local inflammatory reaction was greater in the animals receiving urinary extract than in those receiving beef liver extract. At times this reaction was so marked as to be followed by necrosis and gangrenous changes. This was more frequent in animals receiving larger doses.

SUMMARY

We believe that our results indicate that the urines of patients with leukemia contain some substance or substances which are extractable and separable by the methods described. Depending upon the method used, these extracts are capable of producing in guinea-pigs a specific lymphoid hyperplasia and infiltration with the carbinol fraction, and myeloid hyperplasia and infiltration with the noncarbinol portion. In either case, the chloroform extraction method produced the most potent fractions. The lesions produced in the animals were clinically and pathologically dissimilar to spontaneous leukemia. Further confirma-

tion of the reciprocal relationship between lymphoid and myeloid tissues was observed.

The question of the relationship of substances such as native proteins, normal butyl succinate, and the carbinol extracts of beef liver to the fractions obtained from the urines of patients with lymphoid leukemia is yet to be defined. Similarly, the relationship of the myeloid stimulators, the nucleic acid derivatives, cottonseed oil, and the non-carbinol extracts of beef liver to the fractions obtained from the urines of patients with myeloid leukemia is of interest.

It is our belief that our data justify continuation of investigation. Efforts to purify and concentrate the active factors involved are being pursued.

Urinary extracts were prepared by Dr. Frank Stirn and Dr. E. C. Yen at the Lederle Laboratories, Pearl River, N.Y.

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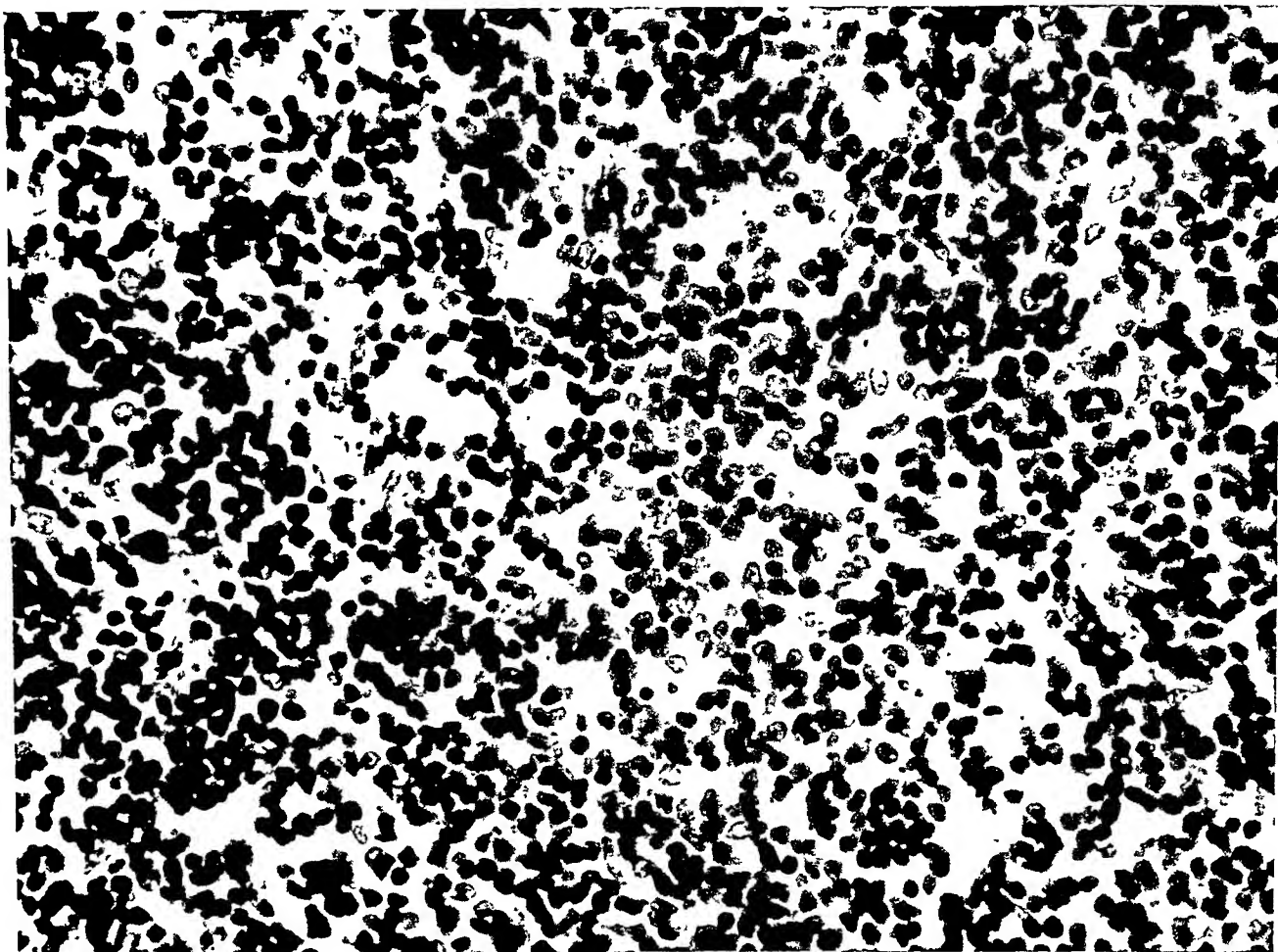
DESCRIPTION OF PLATES

PLATE 180

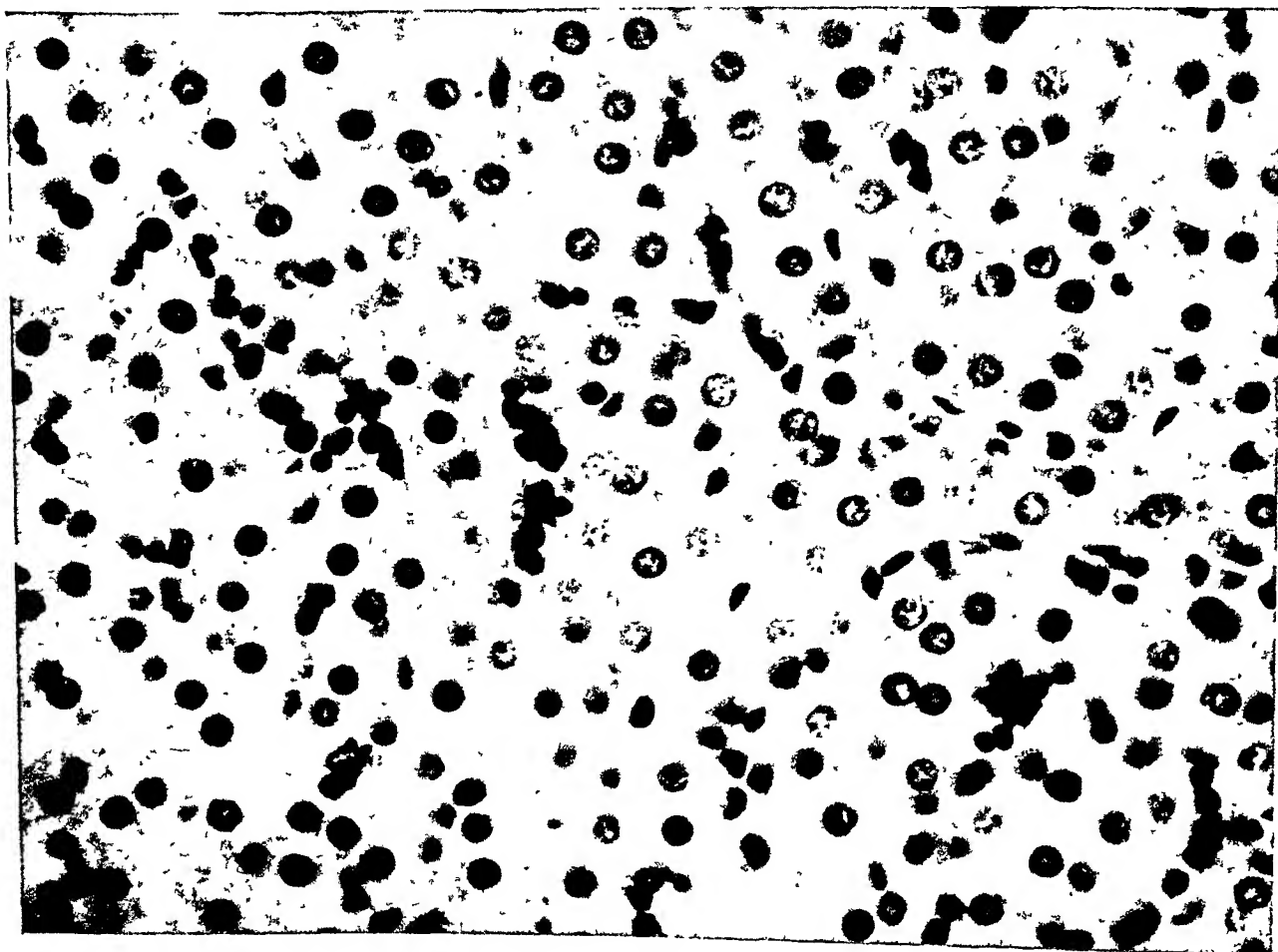
FIG. 1. Lymphoid hyperplasia in cervical node. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) $\times 500$.

FIG. 2. Lymphocytic infiltration in liver. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) $\times 500$.

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2



Sawitsky and Meyer

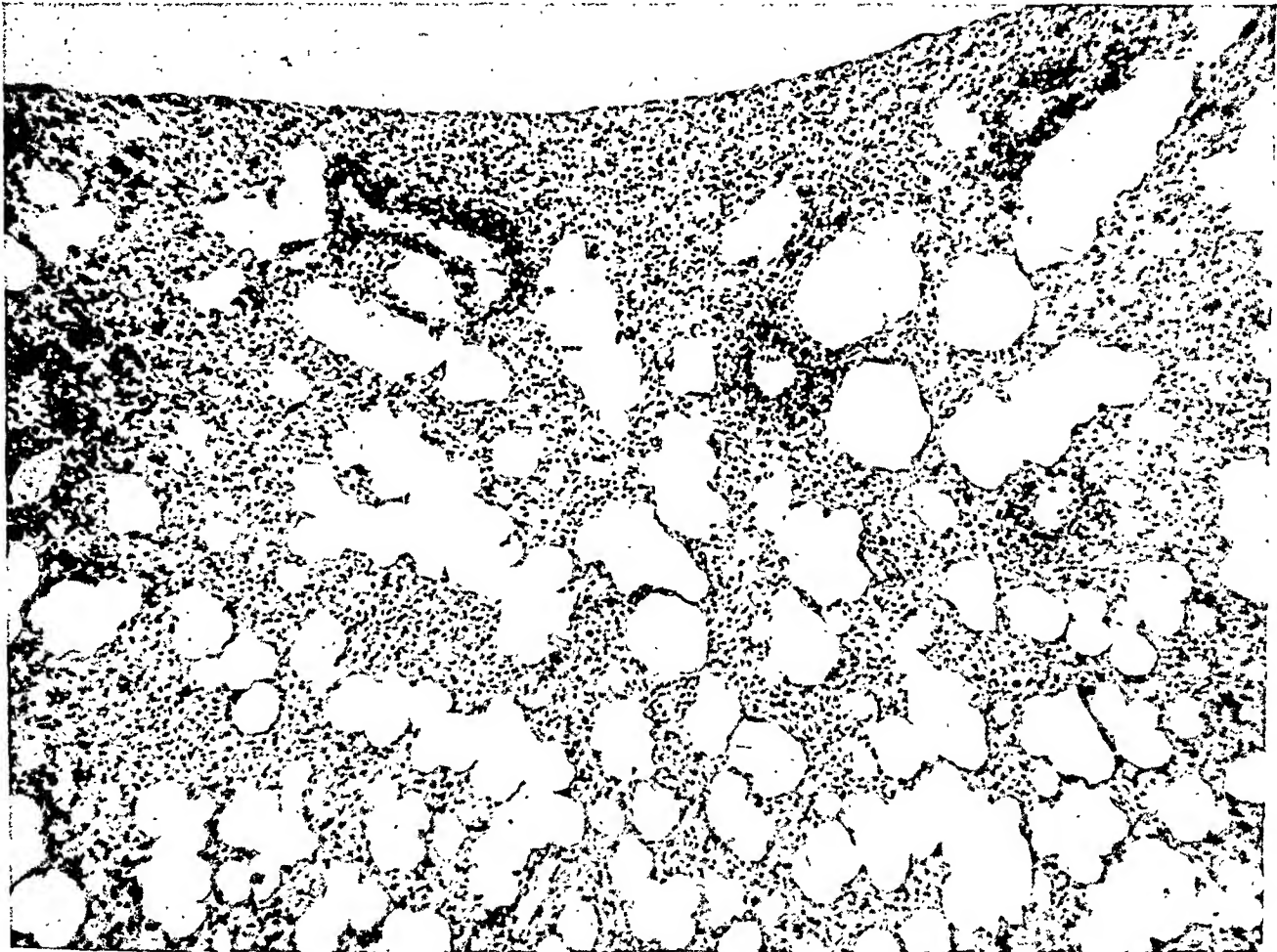
Hematopoietic Substances Derived from Urine

PLATE 181

FIG. 3. Perivascular lymphocytic infiltration in lung. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) $\times 100$.

FIG. 4. Lymphocytic hyperplasia in spleen. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) $\times 500$.

3



4

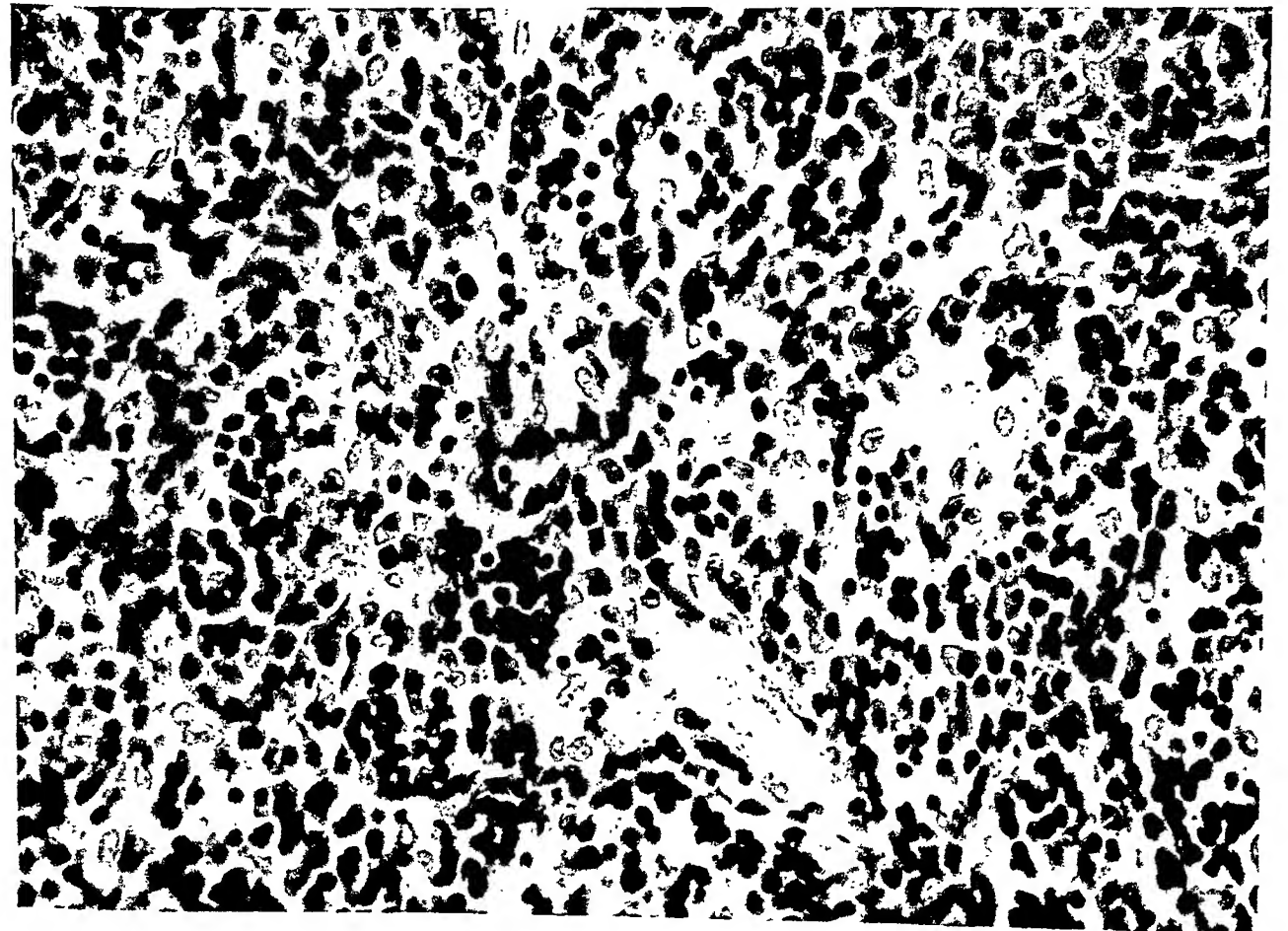
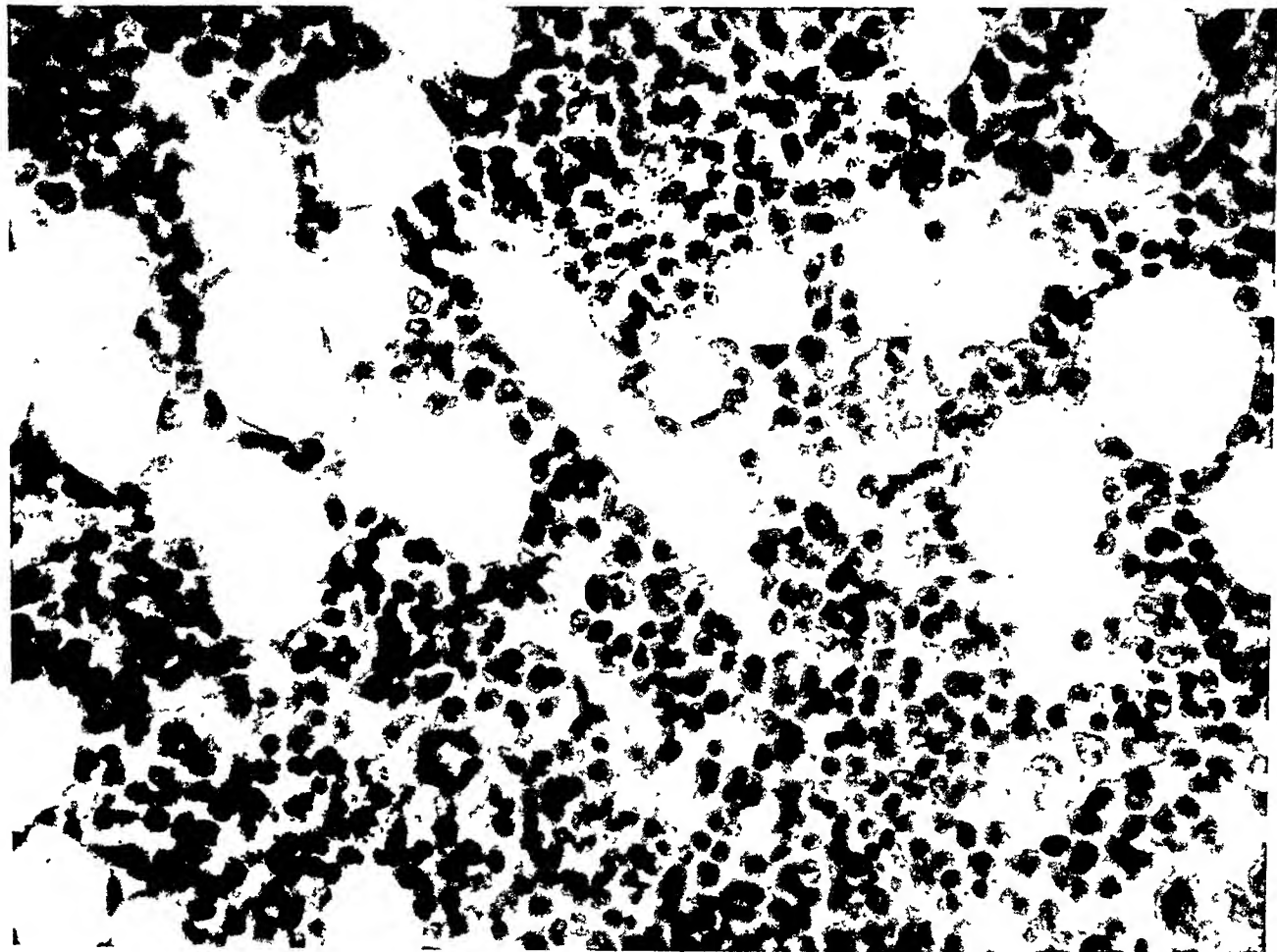


PLATE 182

FIG. 5. Normal bone marrow. $\times 500$.

FIG. 6. Lymphocytic infiltration in bone marrow. (Carbinol fraction prepared from the urines of patients with lymphoid leukemia.) $\times 500$.

5



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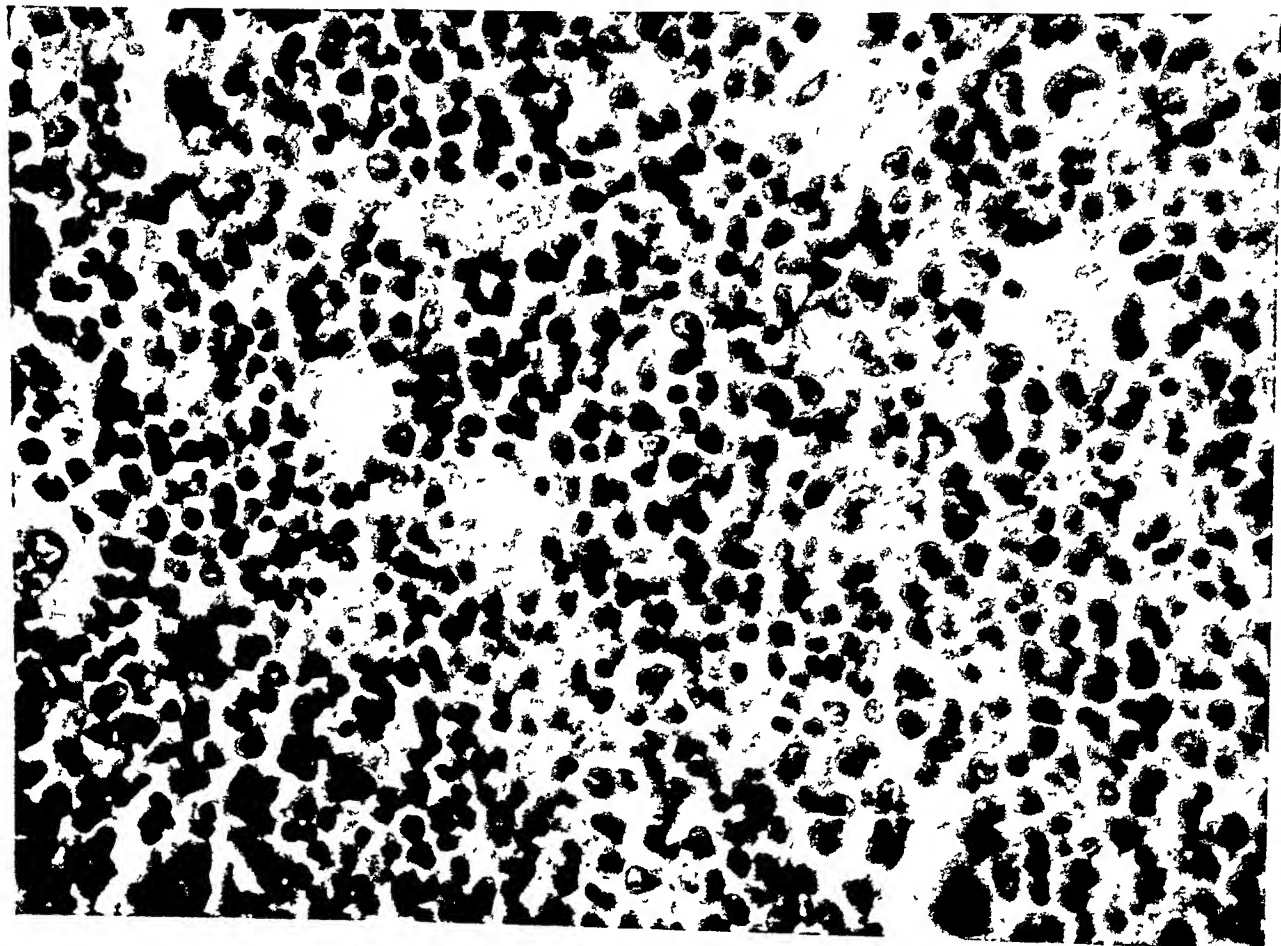
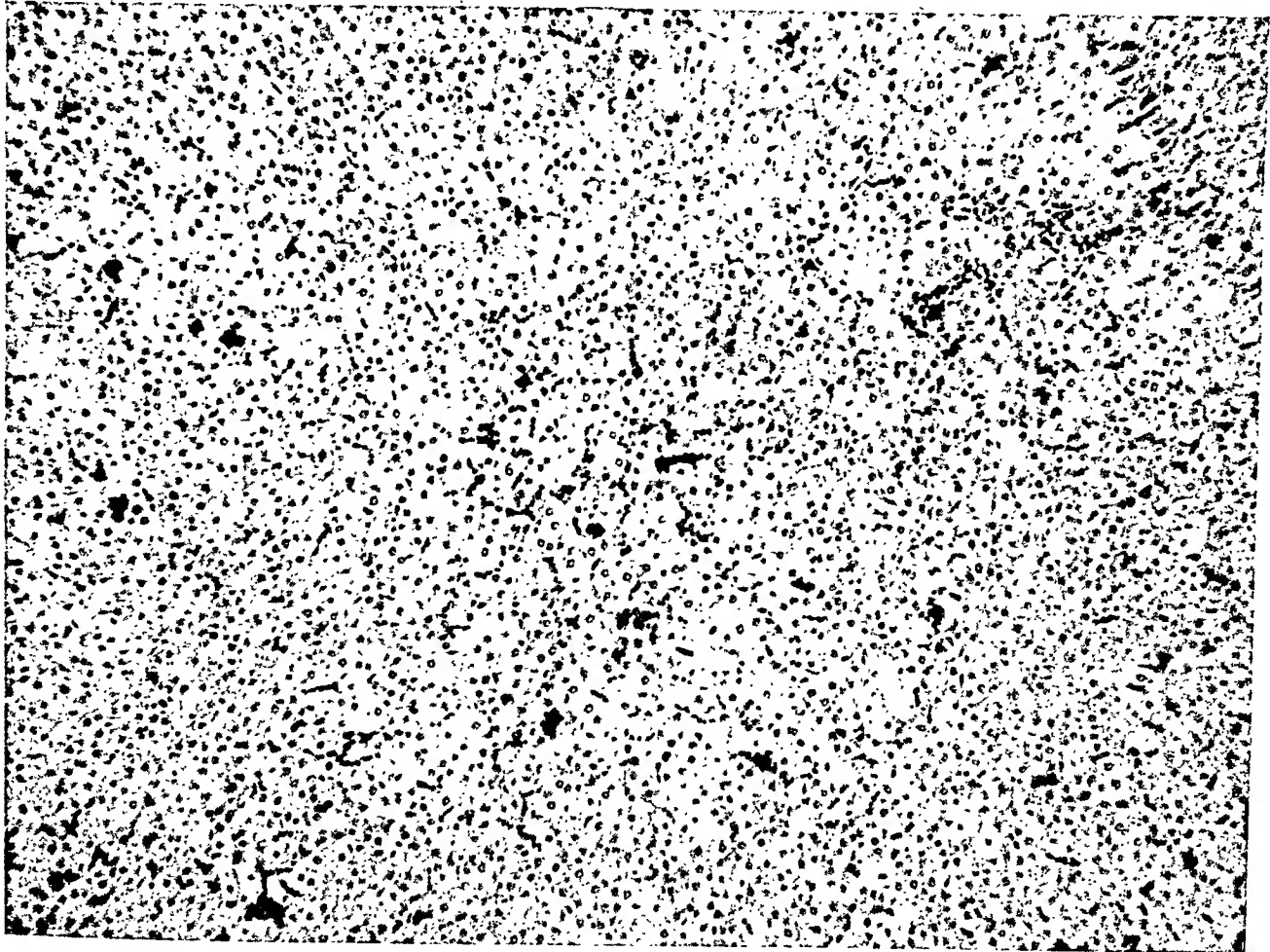


PLATE 183

FIG. 7. Myeloid infiltration in liver. (Noncarbinol fraction prepared from the urines of patients with myeloid leukemia.) $\times 100$.

FIG. 8. Myeloid infiltration in liver. (Noncarbinol fraction prepared from the urines of patients with myeloid leukemia.) $\times 500$.

7



8

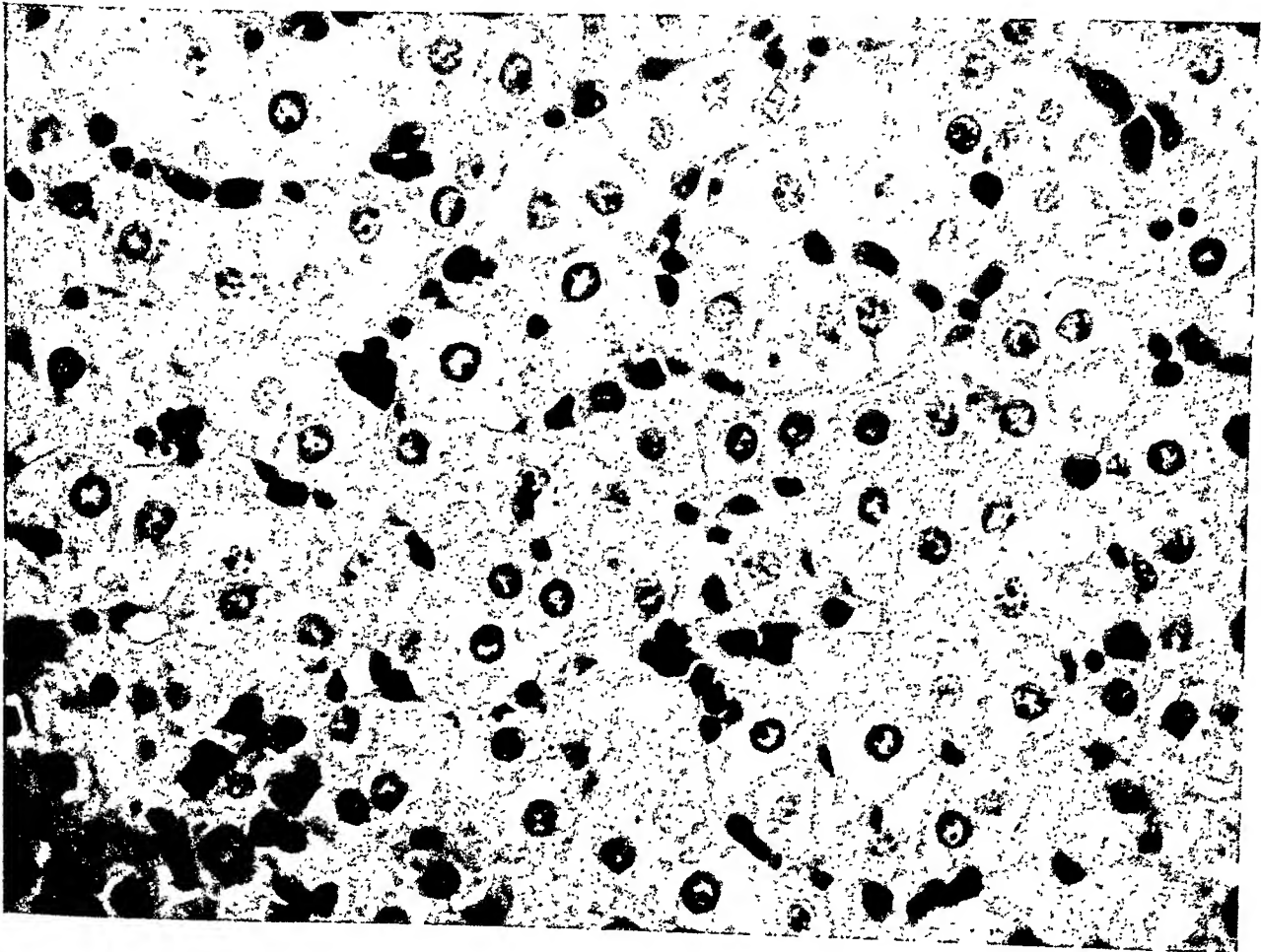
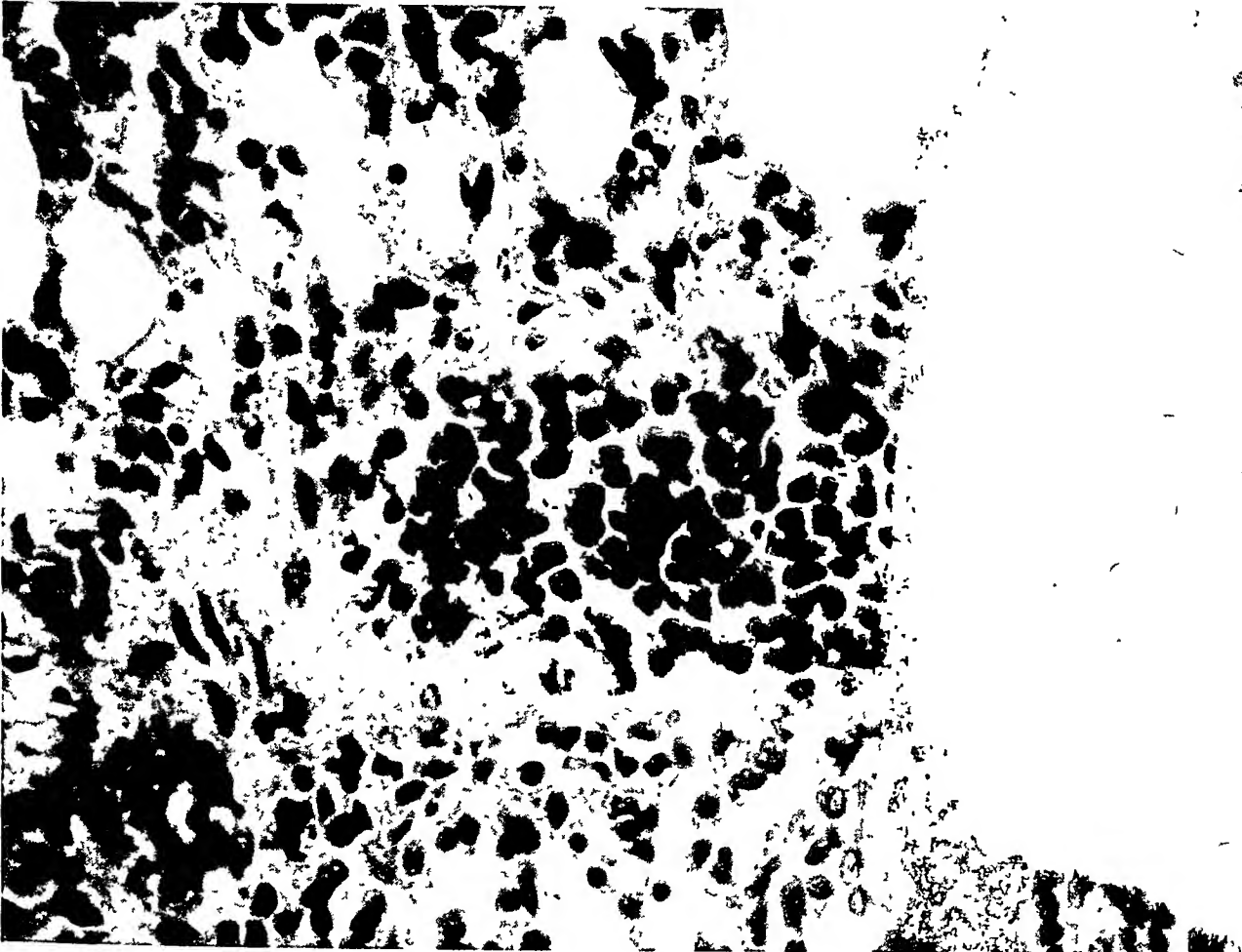


PLATE 184

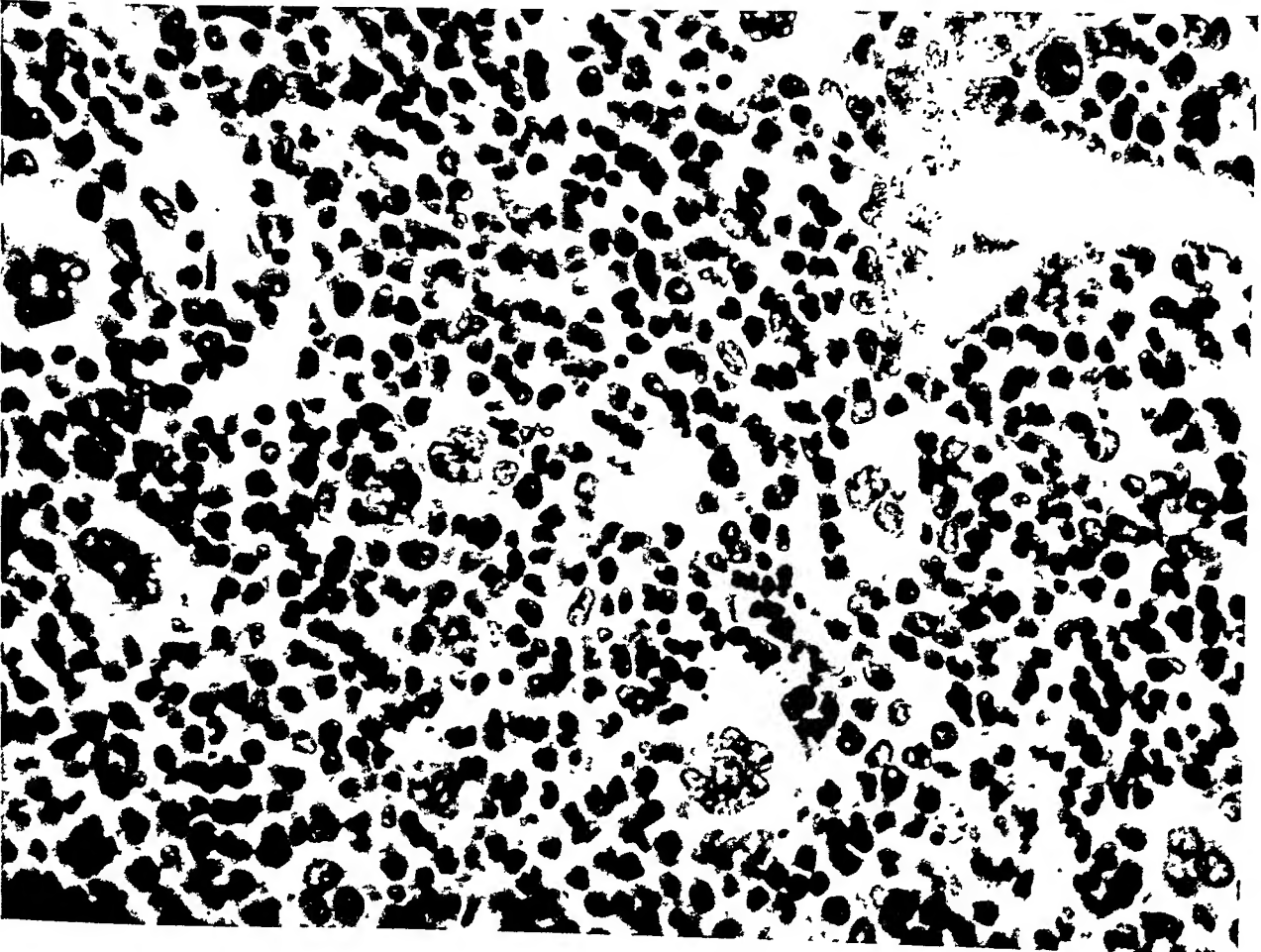
FIG. 9. Plug of myeloid cells in pulmonary arteriole. (Noncarbinol fraction prepared from the urines of patients with myeloid leukemia.) $\times 500$.

FIG. 10. Myeloid hyperplasia in bone marrow. (Noncarbinol fraction prepared from the urines of patients with myeloid leukemia.) $\times 500$.

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THE PNEUMONITIS AND GRANULOMATOSIS PECULIAR TO BERYLLIUM WORKERS *

FRANK R. DUTRA, M.D.

(From the Kettering Laboratory of Applied Physiology, College of Medicine, University of Cincinnati, Cincinnati, Ohio)

The pathologic aspects of the acute pneumonitis and chronic pulmonary granulomatosis of beryllium workers have been discussed briefly in several publications.¹⁻⁴ There has been no previous attempt to describe the changes in the tissues of groups of cases representing both acute pneumonitis and granulomatosis. This report has been compiled following a study of tissues from the bodies of 7 persons who died of acute pneumonitis and of 13 persons who died of chronic granulomatosis.

BERYLLIUM AS A PATHOGENIC AGENT

There is evidence that beryllium is not an innocuous substance when in contact with living tissues. This has been recognized in beryllium-using industries for several years, in which it was observed that the accidental introduction of beryllium compounds beneath the skin resulted in chronic ulcers which persisted until the tissues containing the metal were excised or until the foreign material was eliminated. Local tissues from 3 cases in which beryllium phosphors † had been accidentally introduced beneath the skin were examined in this laboratory. The similarity of the lesions in these subcutaneous sites to each other, and to the granulomas which were found in the lungs of persons who died of beryllium granulomatosis, was so marked as to provide fortuitous but highly advantageous evidence of the pathogenicity of beryllium.

Further evidence that beryllium in the tissues stimulates an inflammatory response has been presented by surgeons who have experimented with metals in repairing bone defects.⁵ It was found that an

* Acknowledgment is made for financial aid from the Sylvania Corporation for the work reported herein.

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† Beryllium phosphors are powders which contain from 4 to 12 per cent beryllium, expressed as beryllium oxide.

alloy containing nickel, cobalt, chromium, and molybdenum produced no tissue reaction, but that the addition of 1.6 per cent of beryllium to the alloy caused the formation of "chronically inflamed granulation tissue containing many macrophages." It was also reported that there was extensive fibrosis with marked lymphocytic infiltration.

BERYLLIUM PNEUMONITIS AND BERYLLIUM PNEUMONOCOCONIOSIS

The clinical aspects of the pulmonary disease peculiar to beryllium workers have caused it to be classified into two mutually exclusive conditions—acute pneumonitis and chronic pulmonary fibrosis—with little clinical evidence of a direct relationship between the two conditions. Acute pneumonitis has been marked by a sudden onset, with cough, chest pain, dyspnea, cyanosis, diminished vital capacity, and loss of weight. The illness lasts from 5 weeks to 4 months and the mortality rate is about 1 per cent. Pulmonary fibrosis (delayed pneumonitis,² chronic pulmonary granulomatosis⁶) has had an insidious onset during which there is increasing weight loss and weakness, cough, and dyspnea. Some of the patients with chronic granulomatosis are alive and working 5 years after the diagnosis was made. About 10 per cent of all patients diagnosed have died. From the standpoint of morbid anatomy this differentiation into acute and chronic diseases is not clear-cut, and all stages of transition have been seen between the conditions designated as acute pneumonitis and as chronic granulomatosis.*

Furthermore, the designation "acute pneumonitis" is inadequate from the pathologic standpoint in some cases which fit clinically into this category, because organization of exudate, which is not ordinarily considered as part of a strictly acute reaction, has been noted in 6 of the 7 cases of acute pneumonitis from which I have had tissues. Similarly, the designation "chronic granulomatosis" is likewise inadequate, because it neglects other important pathologic aspects of the clinically chronic disease. Emphysema and diffuse fibrosis are presumably much more important, from the standpoint of the well-being and pulmonary function of the patient, than are the granulomas.

The development of granulomas has been studied through a number of the transitions in the lungs of various patients. The earliest phase

* After this paper was submitted, it was brought to my attention that at the Greater New York Safety Conference, April 13-16, 1948, Dr. Willard Machle stated that "the differentiation between acute, subacute, and chronic disease from beryllium is purely arbitrary. Our own experience indicates that all gradations in rate of onset, severity, and resolution on a time base may be seen." The differences, Dr. Machle pointed out, are probably related to the solubility of the compounds to which the patients were exposed.

of a recognizable granuloma has been seen in a patient who died of acute beryllium pneumonitis. The well developed granulomas have all been confined to patients who died of chronic granulomatosis.

Tissues from 10 patients who died of beryllium pneumonitis or granulomatosis have been analyzed for beryllium by the method of Cholak and Hubbard.⁷

Table I is presented as a guide to the cases which form the basis of this report upon the pathologic aspects of pulmonary disease of beryllium workers. The results of analyses of the lung tissues are included also.

GROSS MORPHOLOGIC FINDINGS

I have made gross examinations of tissues from 5 persons who died of acute pneumonitis of beryllium workers. In these cases the basic pulmonary lesions have been similar. The lungs have been heavy, the combined weight of both lungs being more than 2,000 gm. in 2 of the 5 cases. The lungs had lost their usual elasticity, so that when the chest cavities were opened they retained their expanded shapes. The cut surfaces were fairly homogeneous, and ranged from pinkish gray to bluish gray. Moderate quantities of thin fluid could be expressed. There was little fluid in the bronchi and bronchioles, and no pus was seen. In each case there was moderate enlargement of the bronchopulmonary and peritracheal lymph nodes, and these were soft and fleshy. In one case there was a partially organized infarct in the lower lobe of the right lung, with a thrombus in an artery at the apex of the infarct. In another case the heart was somewhat flabby and slightly dilated. In all cases of acute pneumonitis, there were hyperemia and parenchymatous degeneration of the viscera.

In the cases of chronic beryllium granulomatosis, also, the lungs have been heavy, usually ranging between 1,500 and 2,000 gm. In one case they weighed 2,400 gm. Although the lungs had lost their elasticity and remained in the expanded position, they did not have the homogeneous character of the lungs in cases of acute pneumonitis. In chronic granulomatosis the lungs contained many small, firm nodules, ranging up to 2 mm. in diameter. Emphysema was present invariably, and in most cases the nodules and enlarged air spaces were scattered diffusely throughout the lung. In a few cases the emphysematous areas were limited to focal regions up to 5 or 6 cm. in diameter, which alternated with masses of dense tissue of similar or larger size. Some lungs had a generalized honeycombed appearance due to emphysema, and occasionally there were subpleural emphysematous blebs. In one case a small hemorrhagic infarct was seen in the lower lobe of the right lung. There were focal fibrous adhesions between the visceral

TABLE I
Cases of Pulmonary Disease in Beryllium Workers

Case number	Clinical type	Duration of illness	Approximate length of exposure	Type of exposure	Micrograms of beryllium in 100 gm. of lung tissue	Characteristics of lesions
H-1	Acute	14 days	40 days	Alloys (BeO)	*	Acute pneumonitis; mononuclear, plasma cell, lymphocytic exudate; early organization
D-1	Acute	16 days	6 yrs.	Ore reduction	220	Acute pneumonitis; edema; mononuclear, plasma cell, lymphocytic exudate; no organization
H-2	Acute	21 days	49 days	Ore reduction	128	Acute pneumonitis; mononuclear, plasma cell, lymphocytic exudate; early organization
C-2	Acute	21 days	90 days	Phosphor	20	Acute pneumonitis; mononuclear cell and lymphocytic exudate; organization of exudate; early granulomas
D-2	Acute	27 days	11 mos.	Ore reduction	9	Acute pneumonitis; mononuclear cells, plasma cells, lymphocytes; early organization and beginning of nodule formation
D-3	Acute	14 days	30 days	Ore reduction	12	Acute pneumonitis; mononuclear, plasma cell, lymphocytic exudate; early organization and granulomas
H-3	Acute	61 days	9 mos.	Alloys (BeO)	*	Mononuclear, plasma cell, lymphocytic exudate; early organization
A-1	Chronic	17 mos.	13.5 mos.	Phosphor	*	Fibrosis, granulomas, slight emphysema; mononuclear cells and lymphocytes; conchoidal bodies; some nodules completely fibrotic
A-3	Chronic	7 mos.	9 mos.	Phosphor	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; no conchoidal bodies
B-1	Chronic	24 mos.	6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies

Table 1 (Continued)

Case number	Clinical type	Duration of illness	Approximate length of exposure	Type of exposure	Micrograms of beryllium in 100 gm. of lung tissue	Characteristics of lesions
B-2	Chronic (accidental death)	28 mos.	6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies
B-3	Chronic		6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies
B-4	Chronic	2 yrs.	6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies
B-5	Chronic		6 yrs.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies; some nodules completely fibrotic
B-6	Chronic	5 yrs.	43 mos.	Alloys (BeO)	*	Fibrosis, granulomas, emphysema; mononuclear cells and lymphocytes; conchoidal bodies; some nodules completely fibrotic
C-1	Chronic	13 mos.	4 yrs.	BeO	12.0	Fibrosis, granulomas, emphysema; mononuclear exudate; conchoidal bodies; some nodules completely fibrotic
E-1	Chronic	3 yrs.	?	BeO	1.7	Fibrosis, granulomas, emphysema; mononuclear exudate; conchoidal bodies; some nodules completely fibrotic
F-1	Chronic	1 yr.	4 yrs.	BeO	78	Fibrosis, granulomas, emphysema; mononuclear exudate; conchoidal bodies
G-4	Chronic	15 mos.	5 yrs.	Neighborhood of ore reduction plant	0	Fibrosis, granulomas, emphysema; lymphocytic and mononuclear exudate; conchoidal bodies
G-5	Chronic	18 mos.	1 yr.	Neighborhood of ore reduction plant	0.93	Fibrosis, granulomas, emphysema; lymphocytic and mononuclear exudate; no conchoidal bodies

*No analysis made.

and parietal pleurae in 5 cases. The peribronchial and peritracheal lymph nodes were moderately firm and enlarged in nearly all cases.

In every case of chronic beryllium granulomatosis there was thickening of the wall of the right ventricle of the heart, with dilatation of this chamber. In most cases this was slight but in some it was marked and a true cor pulmonale was present. In addition, there were evidences of chronic passive hyperemia of the abdominal viscera in these cases. There were no renal calculi in the cases reported here, but it is probably significant that such calculi from 2 persons with chronic granulomatosis have been analyzed at the Kettering Laboratory and were found to contain beryllium.

HISTOPATHOLOGIC FINDINGS

Lungs

The lungs in the 7 cases of *acute pneumonitis* of beryllium workers were characterized by inflammatory exudate and the early formation of new connective tissue.

In the earliest phase of the disease, the exudate was comprised of fluid and cells. In some regions, the fluid was seen as homogeneous eosinophilic material, but in other places considerable quantities of fibrin were present also. This fluid filled the lumina of some alveoli, while other alveoli contained relatively small amounts of it. Large mononuclear cells were predominant in the exudate in all acute cases, and among them were scattered lymphocytes and plasma cells in moderate numbers (Fig. 1). These cells, together with a few polymorphonuclear leukocytes and occasional erythrocytes, comprised the only formed elements within the alveolar lumina. The large mononuclear cells had pale reticulated nuclei and pink, finely granular cytoplasm. Many of them had fine granules or fragments of disintegrating cells in their cytoplasm. In the cases of shorter duration, only a few of these cells were vacuolated, but in cases with a duration of 21 days or more, large numbers of the mononuclear cells contained clear vacuoles, so that the cytoplasm appeared foamy. These vacuoles contained lipid material which stained bright orange with sudan IV. The cells lining the alveoli were swollen and some contained mitotic figures. Transition of the lining (septal) cells to free mononuclear clasmatocytes was well represented in all cases of acute pneumonitis.

There were only rare polymorphonuclear leukocytes in the exudate in 6 of the 7 cases of acute pneumonitis. In one case there was a recent pulmonary infarct in which polymorphonuclear leukocytes and erythrocytes were numerous; elsewhere the exudate in this case was like that of the other 6 cases.

In the lumina of some alveoli, there were masses of degenerating

large mononuclear cells. The cells lining these alveoli were invariably enlarged, and in various stages of desquamation. Often there was coalescence of cytoplasm of the desquamated cells to form giant cells around degenerating and necrotic débris. In later stages of this process, there was proliferation of fibroblasts within adjacent alveolar walls, with organization of the periphery of the mass and formation of a granuloma within the lumen of the alveolus (Fig. 3). Silver impregnation revealed early formation of reticulum in the peripheral portions of these masses, with centripetal strands extending toward the centers.

The central débris of the nodules assumed a brightly eosinophilic, homogeneous appearance, characteristic of the ill defined substance or substances called fibrinoid. This fibrinoid substance was stained bright pink in phloxine and methylene blue preparations, green in Masson's trichrome, yellow in van Gieson's, and did not impregnate with silver. The granular débris from which it seemed to be condensed stained blue with phloxine and methylene blue, and green with Masson's trichrome method.

Although the amount of fibrinoid material was small in the majority of cases of acute pneumonitis, it was found in 6 of the 7 cases. In one case it was abundant, and was usually found in regions where clasmato-cytes were numerous. It lay free in the alveolar spaces, and usually had a serpentine appearance, although occasionally it formed irregular strands or globules. Often it was partly or completely surrounded by large clasmatocytes which sometimes were fused to form multinucleated giant cells. Where fibroblasts and lymphocytes formed nodules in the walls of septa, masses of fibrinoid substance were often found associated with them (Fig. 4).

The origin of this fibrinoid material is not known, but in one case there was a suggestion of transitional stages from degenerating large mononuclear cells, through slightly eosinophilic granular débris, to increasing condensation, homogeneity, and eosinophilia, and, finally, to the fully homogeneous and markedly eosinophilic mature hyalin.

Hyaline membranes lining occasional alveoli and respiratory bronchioles were found in 2 cases of acute pneumonitis. They were affected by stains in the same way as the fibrinoid material, and presumably represented a vagary of distribution of that substance.

In all cases there was infiltration of the interstitial tissues of the septal walls by lymphocytes and plasma cells. These were relatively few in cases of shorter duration, and were numerous in the lungs of those who survived longer, in which they occasionally were grouped with fibroblasts and reticulum into nodules. Similar cells were present in corresponding numbers in the peritruncal connective tissue.

Fibrosis occurred fairly early in acute pneumonitis. This was mani-

fested by early appearance of reticulum in the intra-alveolar exudate and the growth of fibroblasts into that exudate (Fig. 6). Diffuse proliferation of fibroblasts within the walls of the septa was seen also, and in 3 of the 7 cases of acute pneumonitis there was actual formation of nodules here and there within the septa. These nodules consisted of fibroblasts, lymphocytes, and plasma cells, with eosinophilic granular débris or fibrinoid masses at their centers. In one case in which the duration of the disease was 14 days, well defined granulomas were fairly numerous, both within the alveolar lumina and within their walls.

The bronchi in the cases of acute pneumonitis had intact epithelial surfaces, and their walls were invaded by a few lymphocytes and plasma cells. The respiratory bronchioles of most cases showed infiltration of their walls by large numbers of lymphocytes and desquamation of their cuboidal lining cells.

It appeared that the pulmonary lesions in the 13 cases of *chronic granulomatosis* of beryllium workers were closely related to those in acute pneumonitis. In fact, from the morphologic standpoint, the cases of chronic granulomatosis represented a further development of the acute pneumonitis. Cellular exudate, so prominent in acute pneumonitis, was also a part of granulomatosis. Unlike the situation in acute pneumonitis, however, intraseptal lymphocytes and plasma cells were much more numerous than were cells free in the alveolar spaces. There were large mononuclear cells within the alveoli in all of the chronic cases, the numbers varying greatly from case to case and in different portions of the lungs in a given case. Polymorphonuclear leukocytes were virtually absent from the lungs, except in one case.* In addition, the lungs of the patients with granulomatosis also had numerous giant cells which are described in detail below.

Extensive regions of emphysema were present in all cases, with attenuation of septal walls and enlargement of air spaces. The capillaries of the septa in the emphysematous regions were narrowed, so that this condition, together with fibrosis and granulomas in other septa and in the peritruncal tissues, tended to impede the pulmonary circulation.

There was marked fibrosis of the lung in all instances of chronic granulomatosis. This fibrosis was distributed in the septa, in the granulomas, and in the perivascular and peribronchial regions. The collagen was relatively dense, and only moderate numbers of fibroblasts were visible. There was an increase in the diameter of the

* One patient with granulomatosis, who died with superimposed acute pneumonia, had many polymorphonuclear leukocytes in his lungs.

capillaries of most septa, so that the enlargement of the septa resulted from both dilatation of the vascular channels and proliferation of connective tissue.

The granulomas appeared to be characteristic of the pulmonary granulomatosis of beryllium workers. In the cases of long-standing chronic disease, they were confined to the enlarged septal walls or to the peritruncal connective tissues. In all cases, there were regions in the lungs where overgrowth of fibrous tissue effaced the normal pulmonary structure, forming fibrous zones which were at least partially comprised of old granulomas. Doubtless, they also received contributions from the newly formed collagen of the alveolar walls and peritruncal zones. Within these fibrous areas, granulomas were often close together, and occasionally two were found to be contiguous and merging (Fig. 8).

The granulomas consisted of a peripheral zone of loose fibrous tissue which surrounded a central region comprised of necrotic, granular, eosinophilic debris, of fibrinoid material, or of a Langhans' giant cell. The central regions and the fibrous zones were usually infiltrated with lymphocytes, and moderate numbers of large mononuclear cells often were found in the fibrous zone. These cells were fibroblasts, and they had oval, reticulated nuclei. They were arranged with their long axes circumferential. Cells of epithelioid type, so prominent in Boeck's sarcoid, were relatively infrequent. Within some granulomas, there were peculiar basophilic structures which were identified as the conchoidal bodies often found in lesions of Boeck's sarcoid (Fig. 9). Many of the granulomas had centers which contained none of the aforementioned materials, but were comprised of slightly eosinophilic, relatively acellular, fibrous tissue. Silver impregnation of granulomas of this type revealed that the argentophilic material had a radial arrangement, suggesting an ingrowth of reticulum toward the center from the outer zones.

The giant cells sometimes were found in the centers of the granulomas, but often they were among the peripheral fibroblasts. Frequently, several were scattered about within one granuloma. These cells were quite like Langhans' giant cells, differing from the foreign body giant cells of acute pneumonitis. The nuclei of these Langhans' cells were mostly reticular, but many were pyknotic. Sometimes the nuclei were polar, sometimes circumferential, and occasionally they were scattered without pattern in a local area in the cell. The number of nuclei in cross sections of giant cells varied from 4 to more than 30. Many of these cells were in various stages of degeneration. Most had

brightly eosinophilic, slightly granular cytoplasm, but many had foamy or vacuolated centers. In 2 cases, there were rare asteroid inclusion bodies similar to those described by Wolbach.⁸

Associated with the granulomas, sometimes enclosed in the cytoplasm of a Langhans' giant cell, were the conchoidal bodies to which reference has been made above. They varied from 20 to more than 100 μ in greatest diameter, and were usually roughly ovoid. Those within giant cells sometimes were so large that only a thin rim of cytoplasm and nuclei surrounded them. Those which were not in giant cells were invariably lying either within fibrous tissue or granular eosinophilic débris, and they had the appearance of having outgrown an enveloping giant cell. This has been seen by Schaumann,⁹ who described the larger ones as lying in débris of giant cells which had "burst." In some cases these bodies were so numerous that nearly every low-power microscopic field had at least one in it. In other cases a few were found only after extensive search. These bodies were seen in 11 of the 13 cases of chronic granulomatosis. The conchoidal bodies are believed to be identical with those of Boeck's sarcoid. They were blue to black in hematoxylin and eosin preparations, red in van Gieson's preparations, and gave a strongly positive reaction for iron. They were not impregnated with silver salts by Laidlaw's method. Their origin is unknown, and, as pointed out by Schaumann, has been the subject of considerable controversy.

The final stage of the granuloma was seen in sections from 5 cases. This was a single nodule, or a coalescence of several nodules, which had become completely fibrotic (Figs. 10 to 13). Superficially, these resembled the nodules of silicosis, but instead of the tissue being more or less regularly whorled, the strands tended to take a circumferential direction. These fibrous nodules showed relatively little hyalinization, as compared to silicosis, and the individual fibrils were fairly well distinguished. Finally, silver impregnation in these cases failed to reveal central, deeply argentophilic material of the type described by Belt.¹⁰

Microscopic examinations of tissues from persons who died of granulomatosis, utilizing ultraviolet illumination, have revealed fluorescent granules in the lungs of some cases. These studies will form the basis of a subsequent report. The lungs of all cases were examined for acid-fast organisms, and none were found.

Alterations of the pulmonary blood vessels included perivascular fibrosis and nodules encroaching on the adventitia in all cases. In one case there were also numerous thrombi in the smaller tributaries of the pulmonary veins, and these showed advanced organization. The intimal

tunics of the larger arteries were thickened in all cases by the presence of large foam cells of the type common in atherosclerosis. This was due presumably to hypertension of the lesser circulation as a result of the narrowing of the capillary beds.

Bronchopulmonary and Mediastinal Lymph Nodes.

The bronchopulmonary and mediastinal lymph nodes in cases of *acute pneumonitis* were hyperemic, and their sinusoids were filled or partially filled by large mononuclear cells. These cells resembled those which comprised most of the pulmonary exudate. They were actively phagocytic, as evidenced by the fact that the cytoplasm of many enveloped débris of disintegrated cells, whole lymphocytes, or partially disintegrated erythrocytes. The majority of these cells had foamy cytoplasm. The follicles showed relatively little change in most instances, but in one case their centers were in various stages of degeneration. These changes ranged from disappearance of single cells or pyknosis of nuclei to complete necrosis of the central regions with conversion of the centers to eosinophilic granular débris. The lymph nodes of 2 patients had small hyalinized masses of connective tissue, which were relatively acellular and presumably represented old, healed, tuberculous lesions.

The bronchopulmonary and mediastinal lymph nodes in *chronic granulomatosis* showed changes which reflected those in the lungs. These were fibrosis, granulomas, and giant cells. In those cases in which conchoidal bodies were present in the lungs, they were found also in the lymph nodes. In the nodes of several patients there were masses of connective tissue which resembled healed tubercles.

Lesions in Other Viscera

The other viscera in the group representative of *acute pneumonitis* were normal, with one exception. The latter individual had marked centrilobular necrosis of the liver, which was so extensive in most areas that the middle zones also were necrotic. The necrotic regions retained the general architectural arrangement of the liver, the parenchymal cells having been converted to granular eosinophilic ghosts. There were numerous leukocytes of the polymorphonuclear type infiltrating many of the necrotic regions, while other lobules with necrotic centers had no infiltrating cells (Fig. 15). In the same patient there were several focal areas of coagulation necrosis in the vertebral bone marrow. The other organs were normal.

The only lesions outside of the respiratory tract and its lymph nodes, resembling the lesions of *chronic granulomatosis*, were found in

the liver of one patient. These consisted of granulomas within the lobules, consisting of masses of fibrinoid material or of loose connective tissue in which were numerous lymphocytes and plasma cells. Occasional granulomas contained Langhans' giant cells near their centers. There was considerable fibrosis throughout this liver. In some places the connective tissue formed strands which gave the liver the appearance of a cirrhosis of peculiar type. There were polymorphonuclear leukocytes in moderate numbers, as well as lymphocytes and plasma cells, scattered throughout the strands of connective tissue. In one other patient, still alive, a biopsy of the liver revealed similar granulomas, and specimens taken for biopsy of cervical and axillary lymph nodes in several other cases have contained granulomas. In every fatal case, there was thickening of the myocardium of the right ventricle with hypertrophy of the muscle fibers. The livers and other viscera in these cases showed evidences of chronic passive hyperemia.

THE SO-CALLED NEIGHBORHOOD CASES

In the vicinity of industrial plants which are extracting or utilizing beryllium, physicians have become conscious of the hazard to health from fumes or dusts which contain this metal. Pulmonary fibrosis occurring in persons living near a beryllium-handling plant has raised the question of the possibility that beryllium was the cause of the fibrosis. Some such cases have already been shown not to be related to beryllium. Tissues from 2 bodies, concerning which the only exposures had been residence in the neighborhood of a beryllium-reduction plant, were submitted to the Kettering Laboratory for study and analysis. The pulmonary lesions were found to be those of beryllium granulomatosis. In one of these cases an attempt to isolate beryllium from the lungs failed, while in the other case it was possible to isolate 0.93 μg . of beryllium per 100 gm. of lung tissue.

DISCUSSION

There is considerable evidence that the conditions described are the result of the inhalation of fumes or dusts which contain beryllium. The fact that compounds of beryllium are capable, in themselves, of producing inflammation and fibrosis has been demonstrated by work at the Saranac Laboratory,^{3,6} as well as by experiments being conducted at the Kettering Laboratory. Lesions identical with those in the lungs of man have not been reproduced in experimental animals, but fibrosis and granulomas which closely resemble human lesions have resulted in the lungs of animals after exposures to beryllium dusts.⁶ The constant recovery of beryllium from the tissues of persons who

have died with either acute pneumonitis or chronic granulomatosis further attests the relationship of this metal to the pulmonary conditions. Its relationship to the specific granulomas is apparent in the cases of cutaneous and subcutaneous granulomas which resulted from accidental introduction of phosphors into these tissues.

Since the position of beryllium on the periodic chart of the elements is among metals which are not regarded as disease-producing, beryllium has been suspected of having unique properties to account for its pathogenicity. A number of physicians who are acquainted with the beryllium problem have speculated on the possibility that radioactivity might account for some or all of its harmfulness. I am not sympathetic with this suggestion since repeated examinations of products from which pathogenic fumes and dusts have been derived have yielded no evidences of radioactivity.

The lesions of acute pneumonitis of beryllium workers are not specific for that condition in their earlier phases. The pulmonary edema and mononuclear exudate are similar to the lesions produced by the inhalation of cadmium fumes,¹¹ and manganese fumes seem to have caused an analogous condition in the lungs of experimental animals.¹² Kneeland and Smetana¹³ have described the lungs in a fatal case of atypical pneumonia, wherein they stated that large mononuclear cells formed the most prominent part of the exudate. Furthermore, there was a marked tendency to proliferation of fibroblasts and laying down of collagen in the alveolar walls and in the perivascular regions. The lesions of the lungs in Q fever¹⁴ are also similar to those of the early phases of acute beryllium pneumonitis. In Q fever, the alveoli contain large clasmotocytes and there is infiltration of the alveolar walls by lymphocytes and plasma cells. Acute beryllium pneumonitis can be differentiated from pneumonia due to Friedländer's bacillus, with its exudate of large mononuclear cells, by bacteriologic methods; in the latter, the organisms can be cultured from the lungs, and they are also readily stained in tissue sections. On the other hand, the history of exposure to beryllium and the recovery of this metal from the urine before death or from the lungs removed at autopsy serve to differentiate beryllium pneumonitis from all other conditions.

With the beginning of the development of granulomas, as early as 2 weeks after the onset of acute pneumonitis, the character of the lesions comes to differ from that of any other pathologic process. Transitional stages, from the nodules in acute pneumonitis to well defined specific granulomas in chronic granulomatosis, are clearly presented in the material reported here. The lesions of chronic granulomatosis of beryllium workers resemble to some extent those of several

other more or less well defined conditions. Among these are Boeck's sarcoid,¹⁵ "acute diffuse interstitial fibrosis,"¹⁶ and pulmonary fibrosis in a man who worked with radioactive metals.¹⁷

Beryllium granulomatosis has been confused with Boeck's sarcoid by clinicians, radiologists, and pathologists. Several persons now believed to have beryllium granulomatosis have been considered previously to be suffering from Boeck's sarcoid on the basis of roentgenograms and biopsies of lymph nodes. Differentiation between these conditions can be made readily by microscopic examination of the tissues. The specific granuloma of sarcoid is comprised characteristically of a mass of fairly compact epithelioid cells which usually surround a central giant cell. The giant cells tend to be large, and to have many (usually about 25 or 30) peripheral nuclei. The entire nodule is surrounded by loose connective tissue in which there are nearly always a number of lymphocytes. There is little or no fibrosis or exudate, apart from that actually related to the granulomas. In pulmonary granulomatosis of beryllium workers, on the other hand, the granulomas only rarely have giant cells at their centers, and these giant cells differ from those in Boeck's sarcoid, in that there is great variation in the number of nuclei (from as few as 4 to more than 30). Typical epithelioid cells are seldom a part of the granulomas. Most of the granulomas in the lungs of beryllium workers have either fibrinoid material or granular necrotic eosinophilic debris at their centers. The granulomas in beryllium disease are infiltrated with moderate numbers of lymphocytes which are found even within the granular central debris. Furthermore, in beryllium disease, there is diffuse intraseptal fibrosis of a type never seen in Boeck's sarcoid. The large mononuclear cells found in the alveolar spaces in beryllium granulomatosis are not seen as a part of Boeck's sarcoid. Tissues from 7 cases of typical Boeck's sarcoid have been analyzed for beryllium at the Kettering Laboratory, and this metal was not found in any of them.

The cases of diffuse pulmonary fibrosis described by Hamman and Rich¹⁶ and the case of pulmonary fibrosis described by Belt¹⁷ do have the diffuse fibrosis of the lungs found in beryllium granulomatosis, but in neither of these diseases have granulomas or other nodules been described. Furthermore, the cases of Hamman and Rich differed from those in the beryllium workers in that there was virtually no exudate in the former.

CONCLUSIONS

Exposures to dust and fumes containing beryllium have resulted in a number of cases of pulmonary disease. These are of two fairly distinct clinical types: an acute pneumonitis and a protracted pulmonary fibrosis and emphysema.

The pulmonary lesions in these two clinical conditions are specific and there is evidence of transition of the pathologic lesions of the acute condition to those of the chronic one.

In the acute pneumonitis there is a boggy, generalized consolidation of lobes, simulating to some extent the stage of gray hepatization in lobar pneumonia. Microscopically, there is diffuse intra-alveolar exudate composed of large mononuclear phagocytes (septal cells) and edema fluid. Intraseptal lymphocytes and plasma cells are also fairly numerous. In one case centrilobular necrosis of the liver has been seen.

With chronic pulmonary fibrosis (granulomatosis), the lungs have been voluminous and emphysematous. Scattered diffusely throughout the lungs were many fine nodules. Enlargement of the right ventricle of the heart was constantly present in these cases. Microscopic characteristics include diffuse interstitial and nodular fibrosis of the lungs together with a peculiar granulomatous reaction.

The granulomas which are typical of beryllium disease are formed in part within alveolar spaces by the organization of exudate, and in part within the septal and peritruncal connective tissues. They are comprised of central regions of fibrinoid material or granular debris, and the central regions are surrounded by a peripheral zone of fibrosis in which there is an infiltration of lymphocytes and plasma cells. The centers of the granulomas are occasionally occupied by giant cells of the Langhans' type. There are conchoidal bodies in most cases, sometimes within giant cells, sometimes lying free in debris or in fibrous tissue.

Granulomas similar to those of the lungs have been found in the skin and subcutaneous tissues into which beryllium oxide or phosphors have accidentally been introduced. These lesions provide further proof of the pathogenic nature of beryllium.

I wish to express my gratitude for clinical data and for the opportunity to examine tissues to Drs. Morris Carmody, J. M. DeNardi, H. S. Martland, H. T. Karsner, J. B. Hazard, and Willard Machle. The photomicrographs were prepared by Mr. J. B. Homan.

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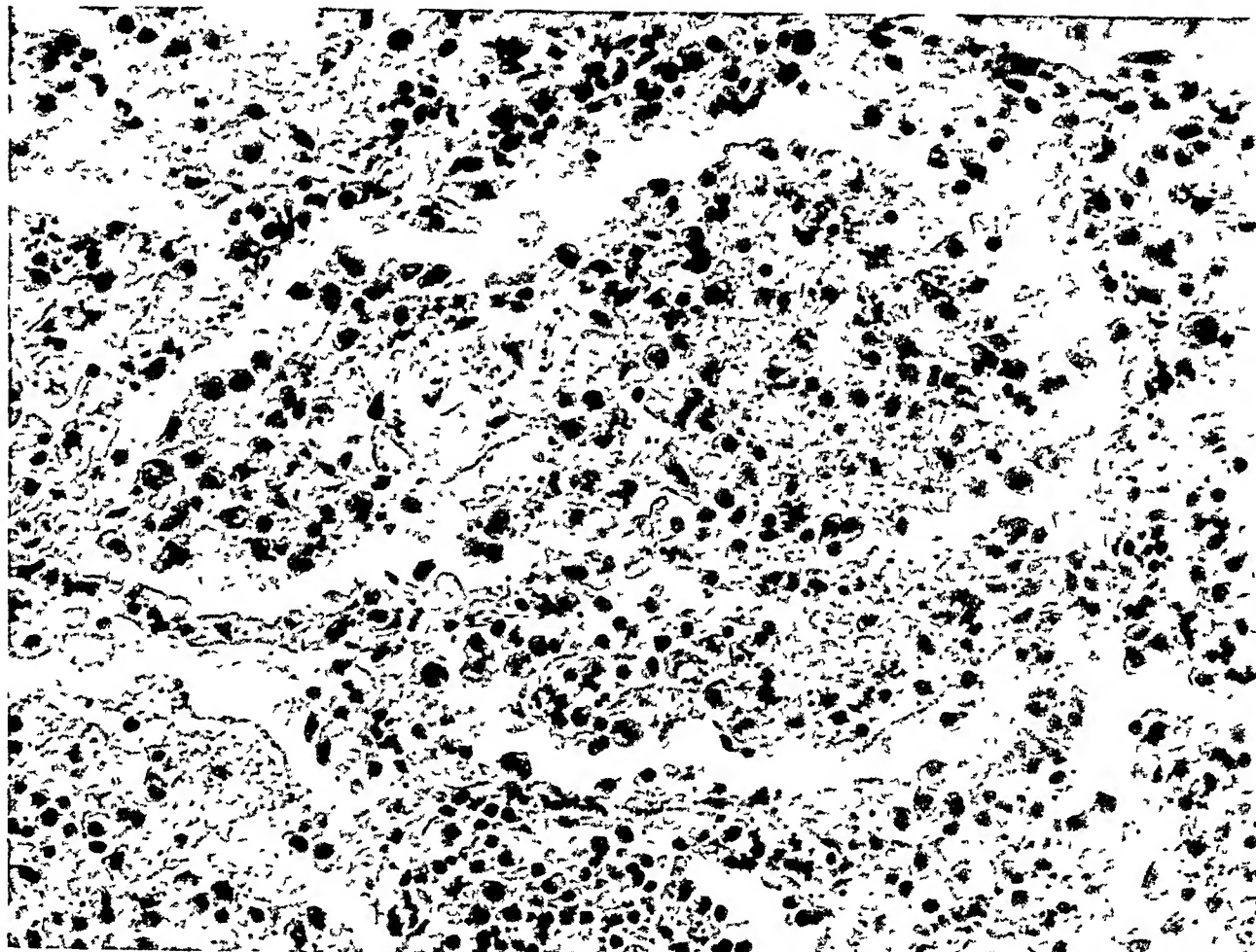
DESCRIPTION OF PLATES

PLATE 185

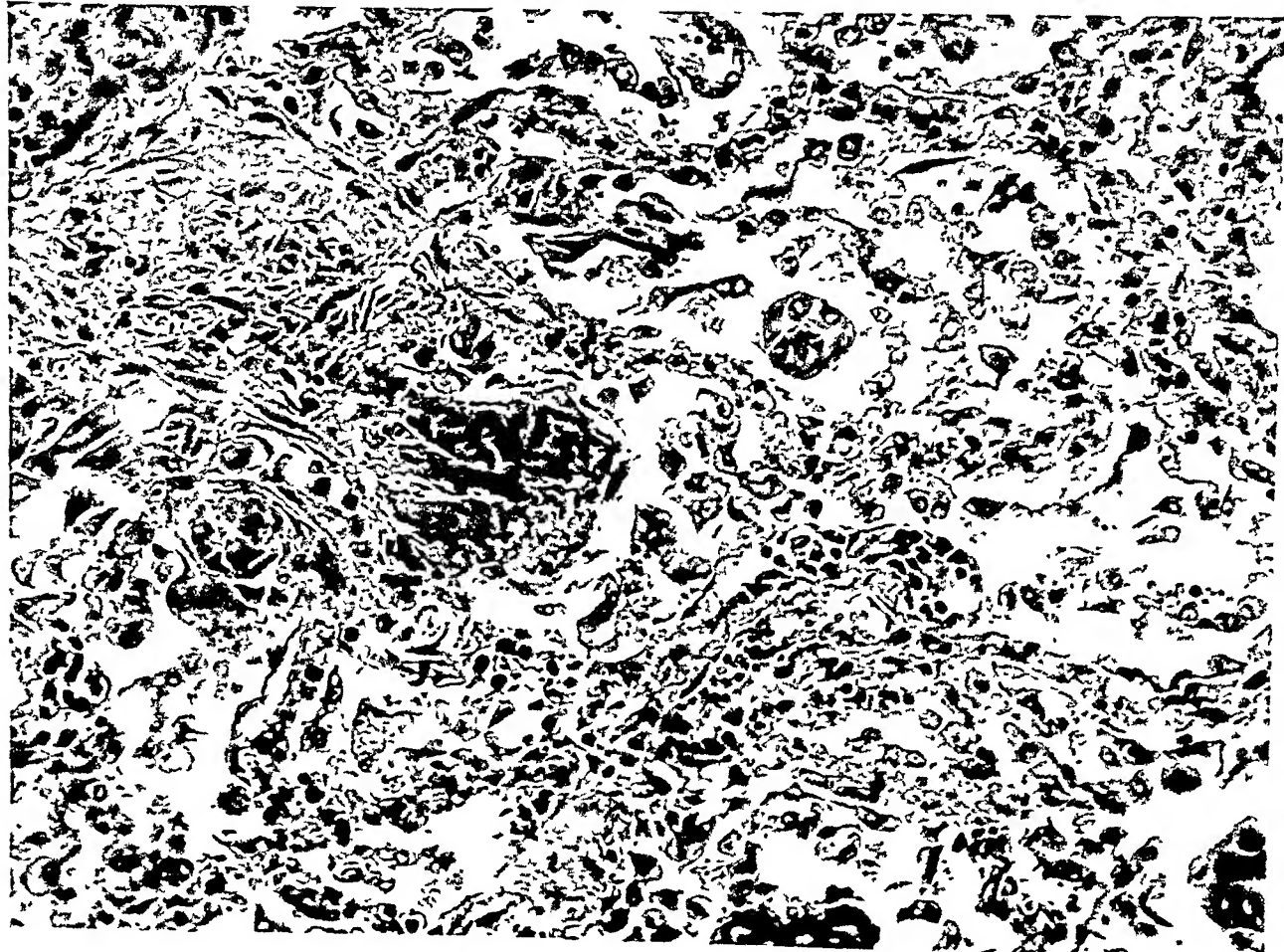
FIG. 1. Exudate comprised of clasmatocytes, lymphocytes, and plasma cells. Acute pneumonitis (case C-2). $\times 320$.

FIG. 2. Early stage of nodule formation. Septal cells desquamating in sheets in uppermost alveoli. Fibrinoid material in nodules. Acute pneumonitis (case D-3). $\times 250$.

1



2



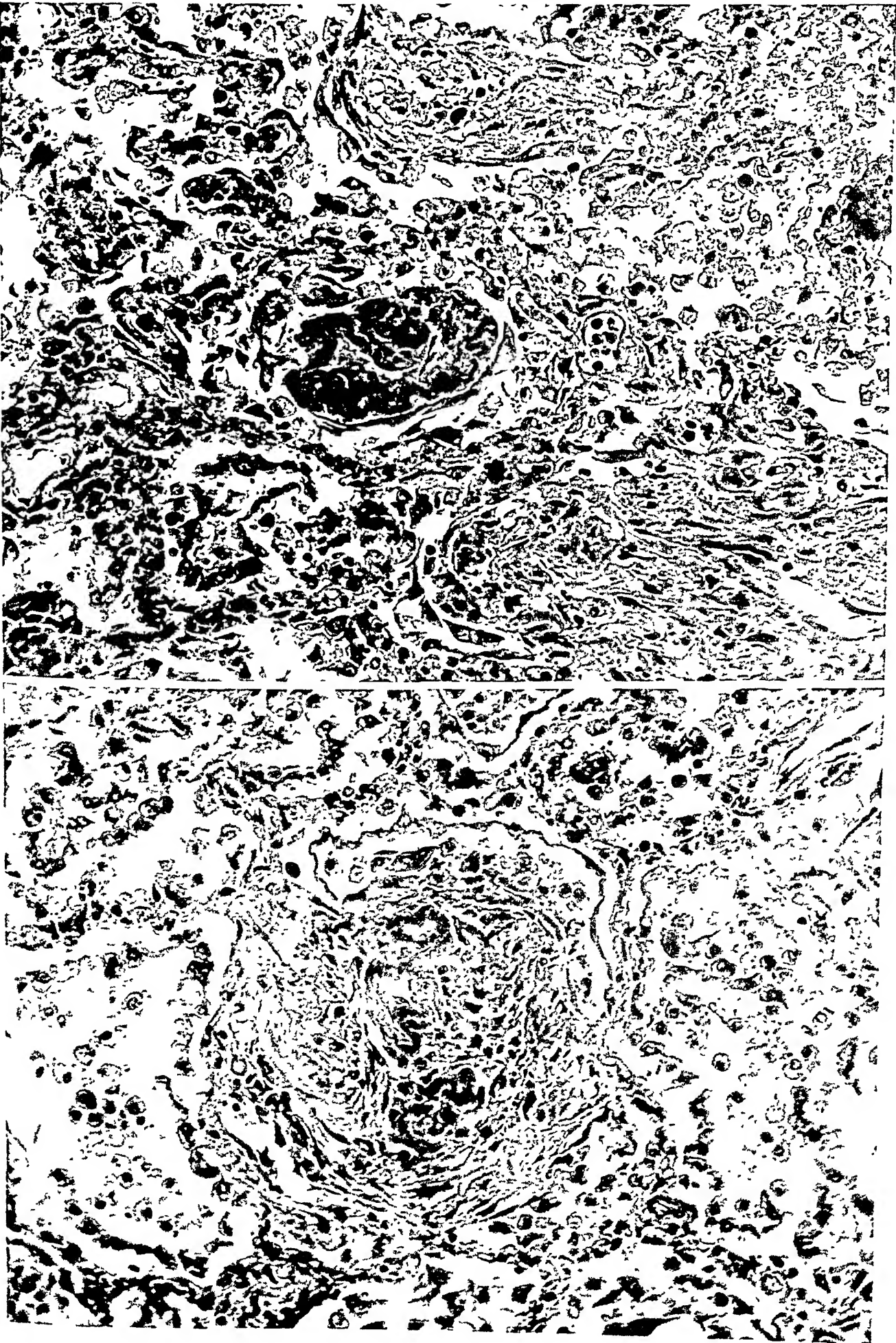
Dutra

Beryllium Pneumonitis

PLATE 186

FIG. 3. Nodule forming within alveolar space. Acute pneumonitis (case D-3).
× 250.

FIG. 4. A well formed granuloma in the wall of the septum. Serpentine fibrinoid material is surrounded by loose collagen. Acute pneumonitis (case D-3).
× 250.



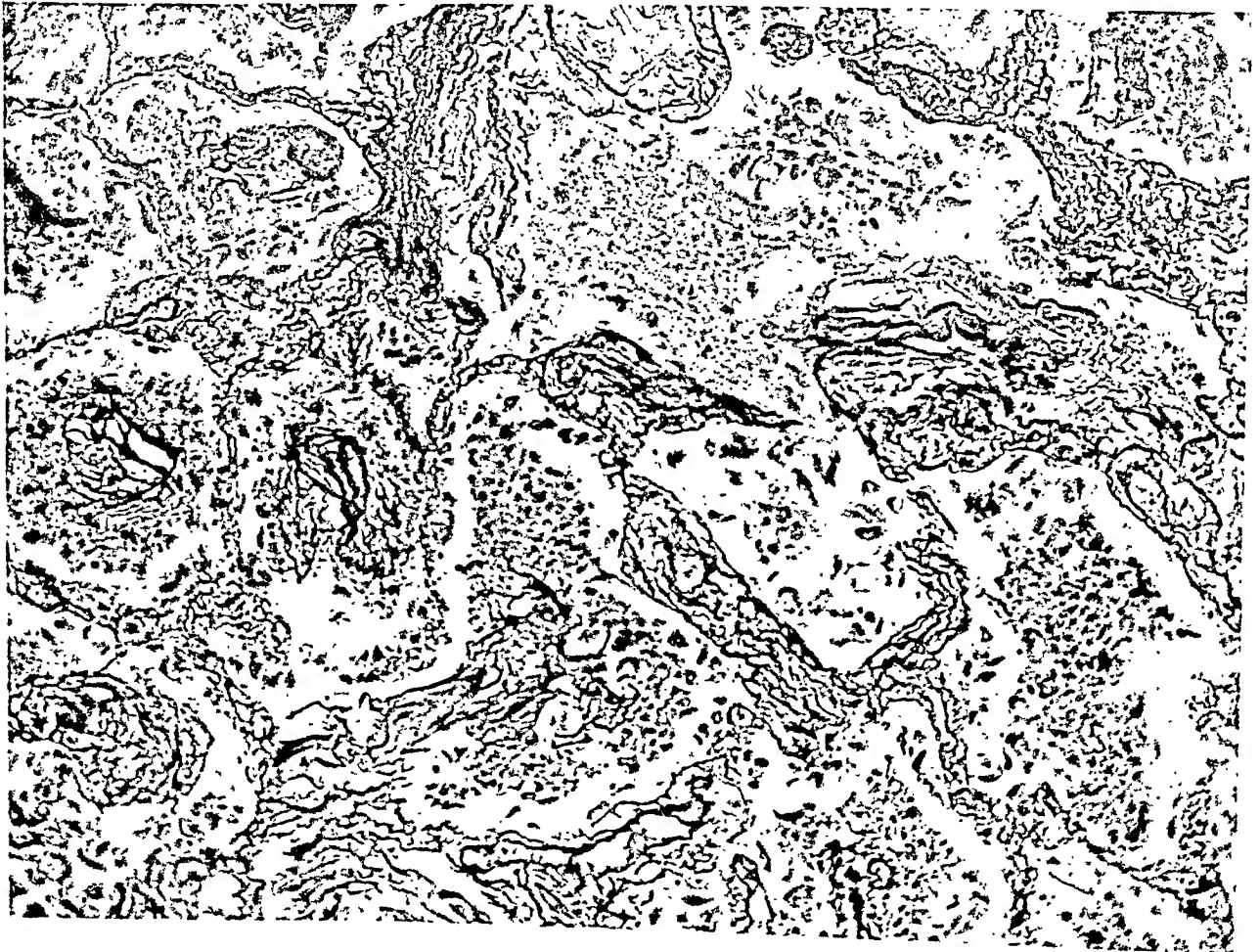
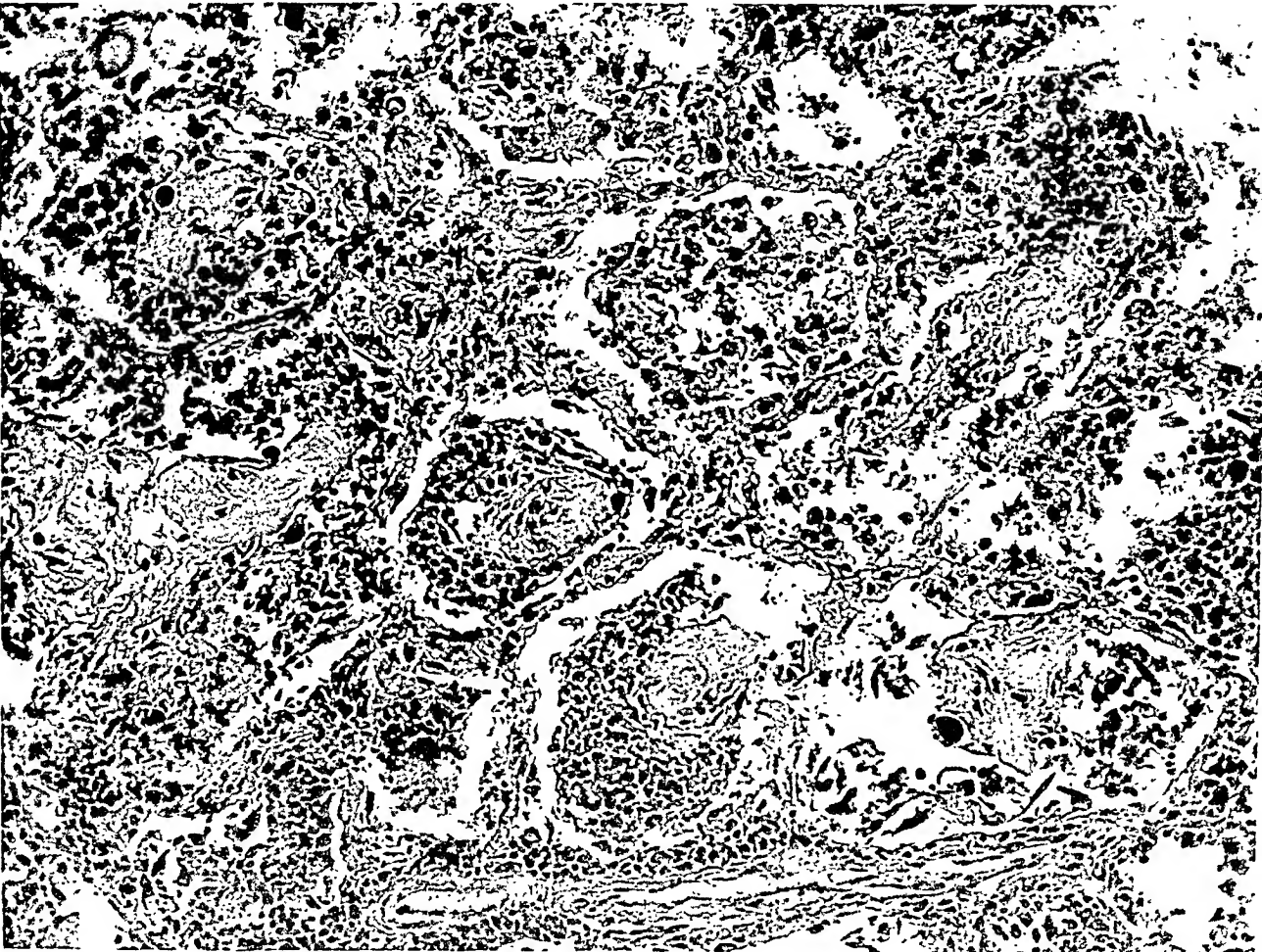
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Beryllium Pneumonitis

PLATE 187

FIG. 5. Advanced granuloma formation and cellular reaction. Acute pneumonitis (case C-2). $\times 160$.

FIG. 6. Silver preparation showing organization of exudate into granulomas and formation of granulomas in septal walls. Acute pneumonitis (case C-2). $\times 160$.



Dutra

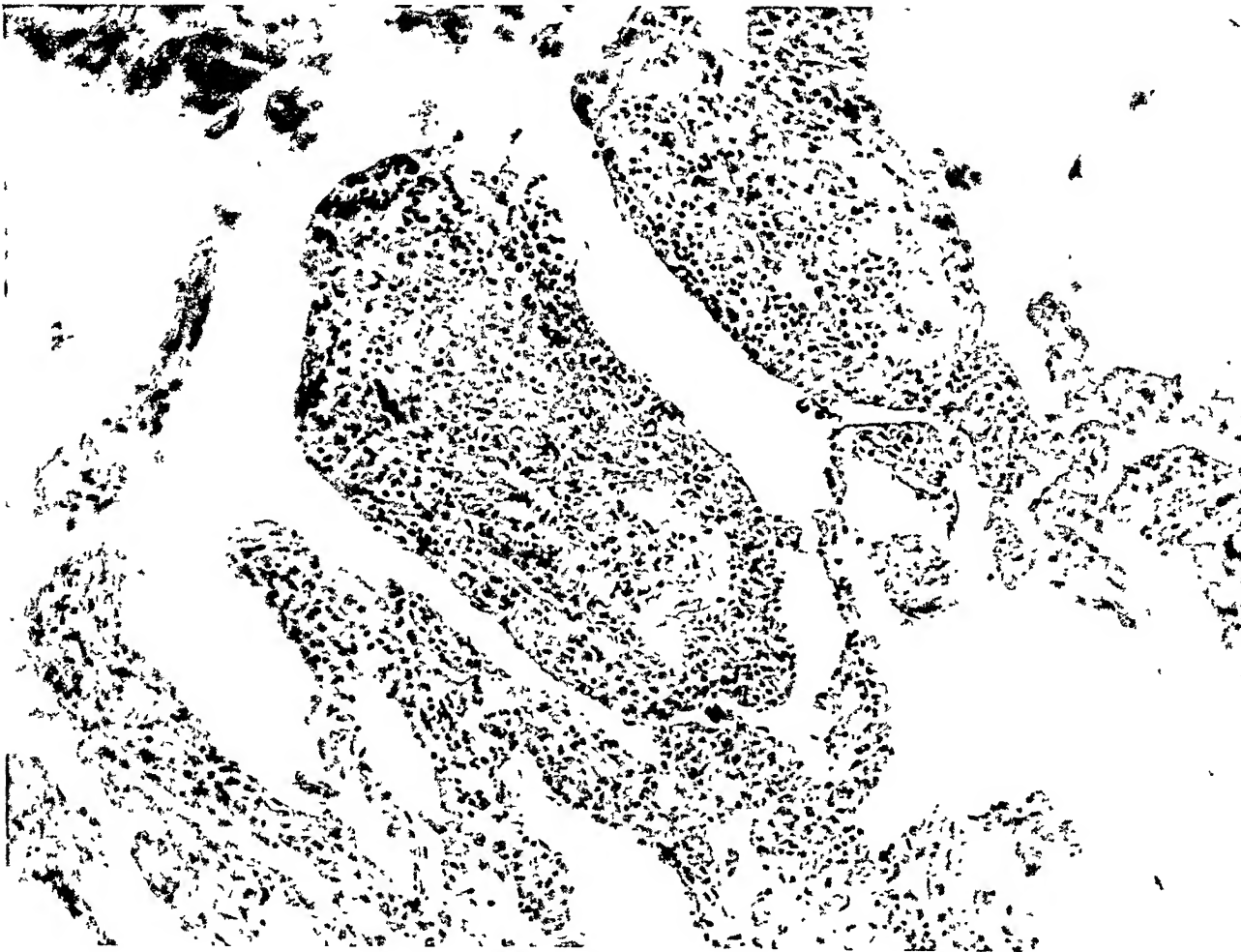
Beryllium Pneumonitis

PLATE 188

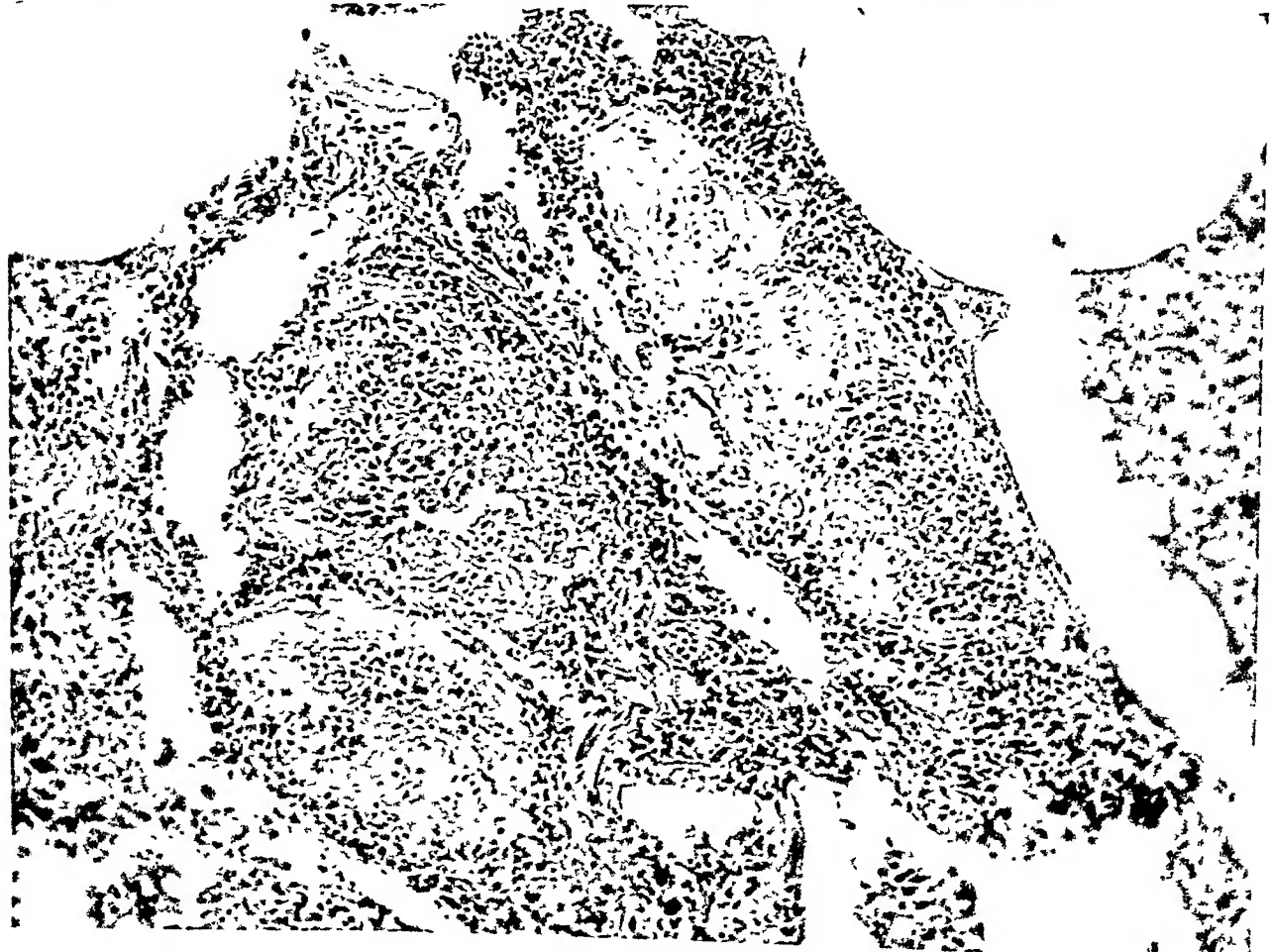
FIG. 7. Two granulomas in alveolar walls. Chronic granulomatosis (case C-1).
× 160.

FIG. 8. Coalescence of granulomas. Chronic granulomatosis (case C-1). × 160.

7



8



Dutra

Beryllium Pneumonitis

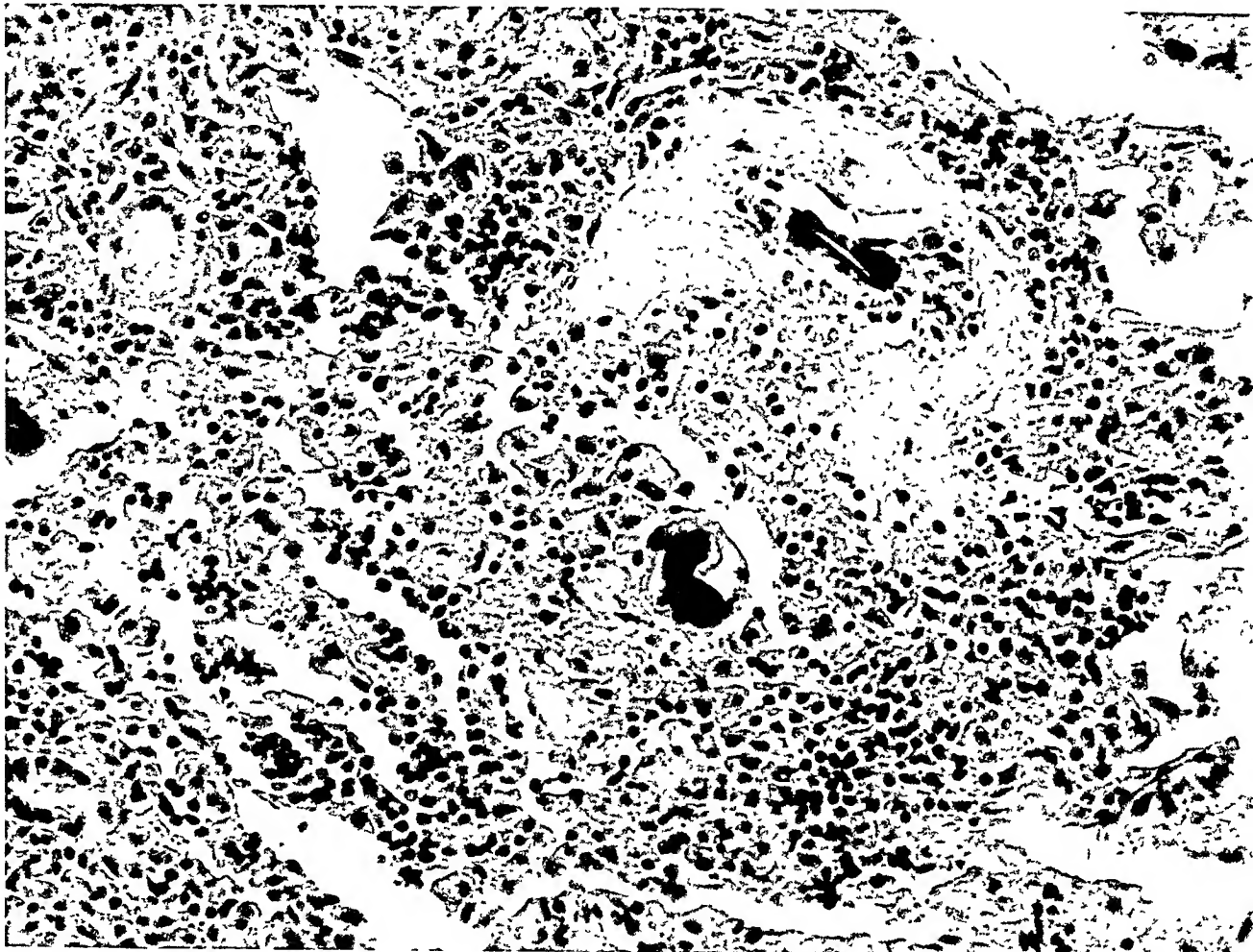
PLATE 189

FIG. 9. Conchoidal bodies. One is within a giant cell and the other lies free in débris within a granuloma. Chronic granulomatosis (case C-1). $\times 320$.

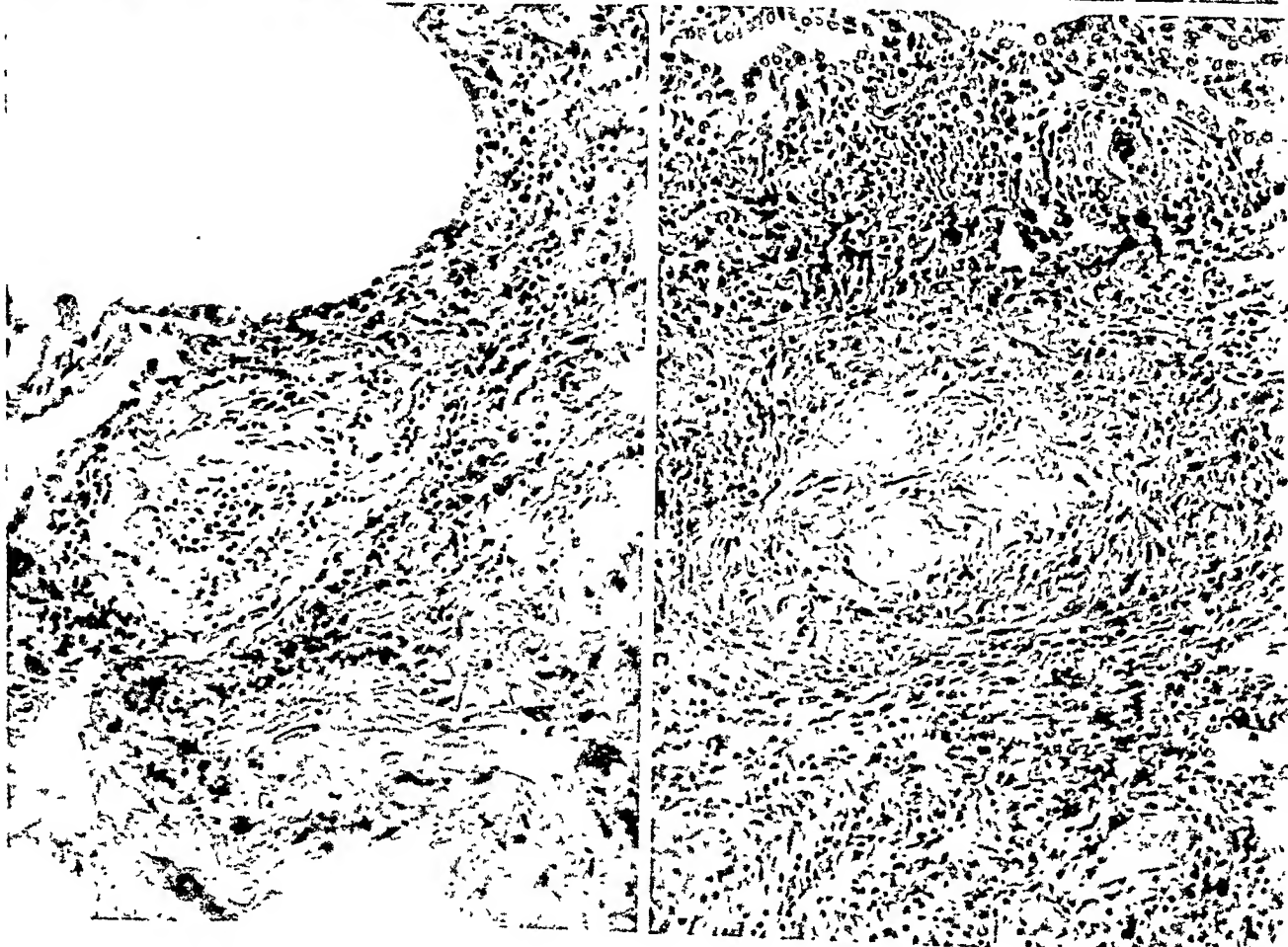
FIG. 10. Fibrosis of a granuloma, early stage, showing thick bands of relatively acellular tissue infiltrated with lymphocytes. Chronic granulomatosis (case C-1). $\times 160$.

FIG. 11. A more advanced stage of fibrosis of a granuloma from a case of chronic granulomatosis (case F-1). $\times 160$.

9



□



Dutra

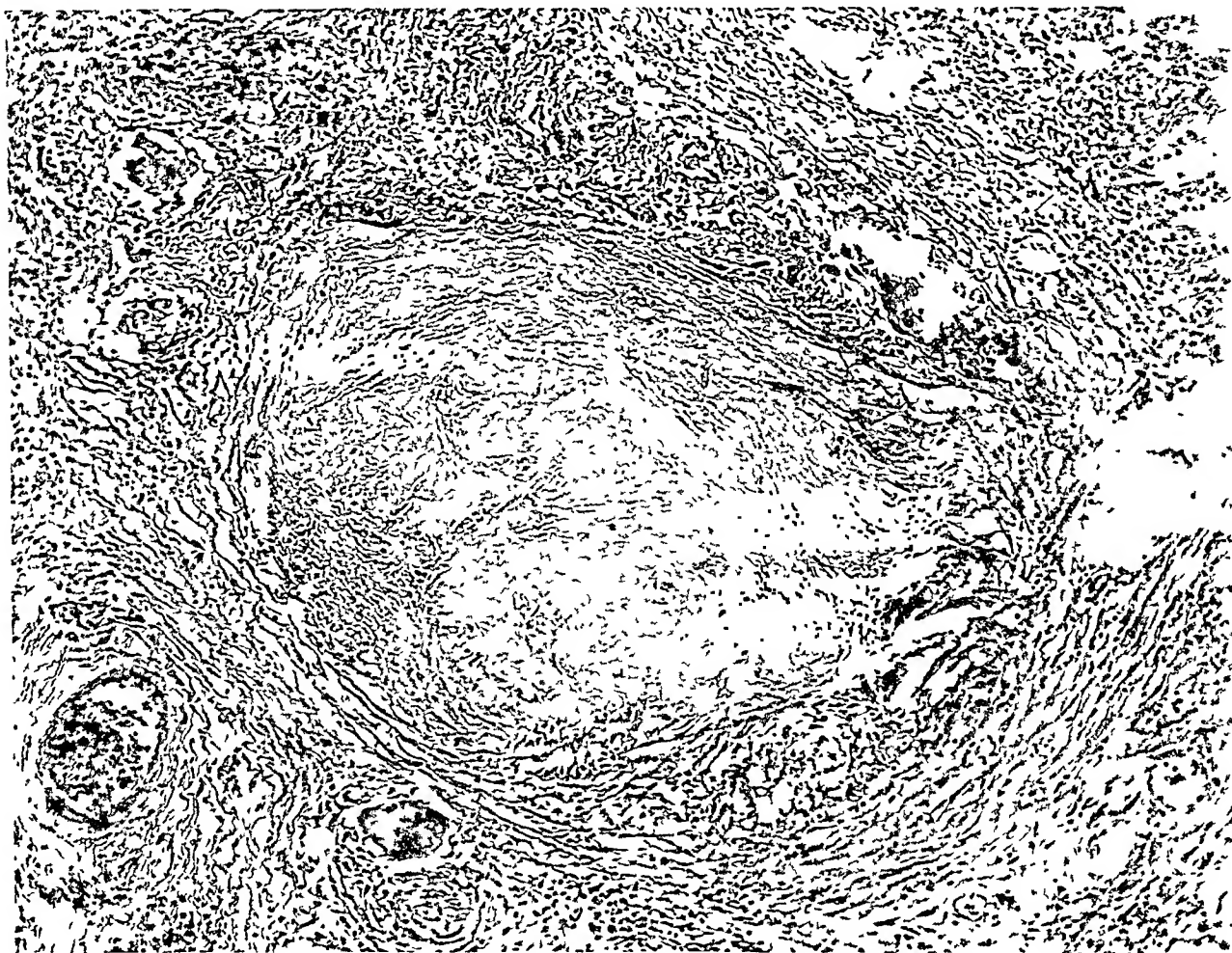
Beryllium Pneumonitis

PLATE 190

FIG. 12. A fibrous nodule surrounded by granulation tissue. The absence of whorls and dense hyalinization differentiate this nodule from those of silicosis. Chronic granulomatosis (case E-1). $\times 125$.

FIG. 13. A conglomerate fibrous nodule. Chronic granulomatosis (case A-1). $\times 85$.

12



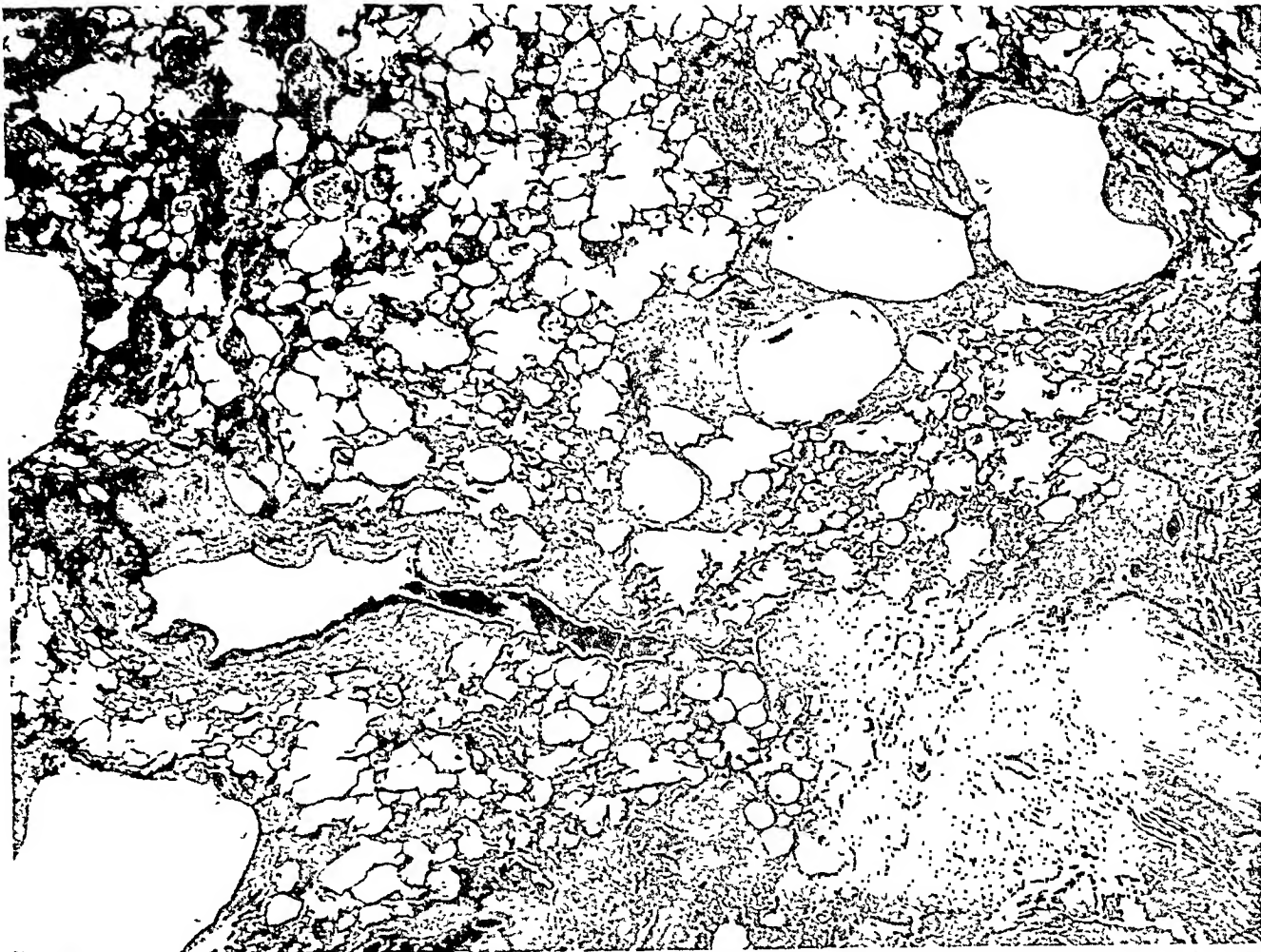
13



PLATE 191

- FIG. 14. Low-power photomicrograph to show the locations of nodules in the perivascular tissues and in the walls of septa, and the marked emphysema associated with chronic granulomatosis (case C-1). $\times 16$.
- FIG. 15. Liver from a case of acute pneumonitis showing centrilobular necrosis—evidence of systemic toxicity of beryllium (case D-3). $\times 160$.

4



15

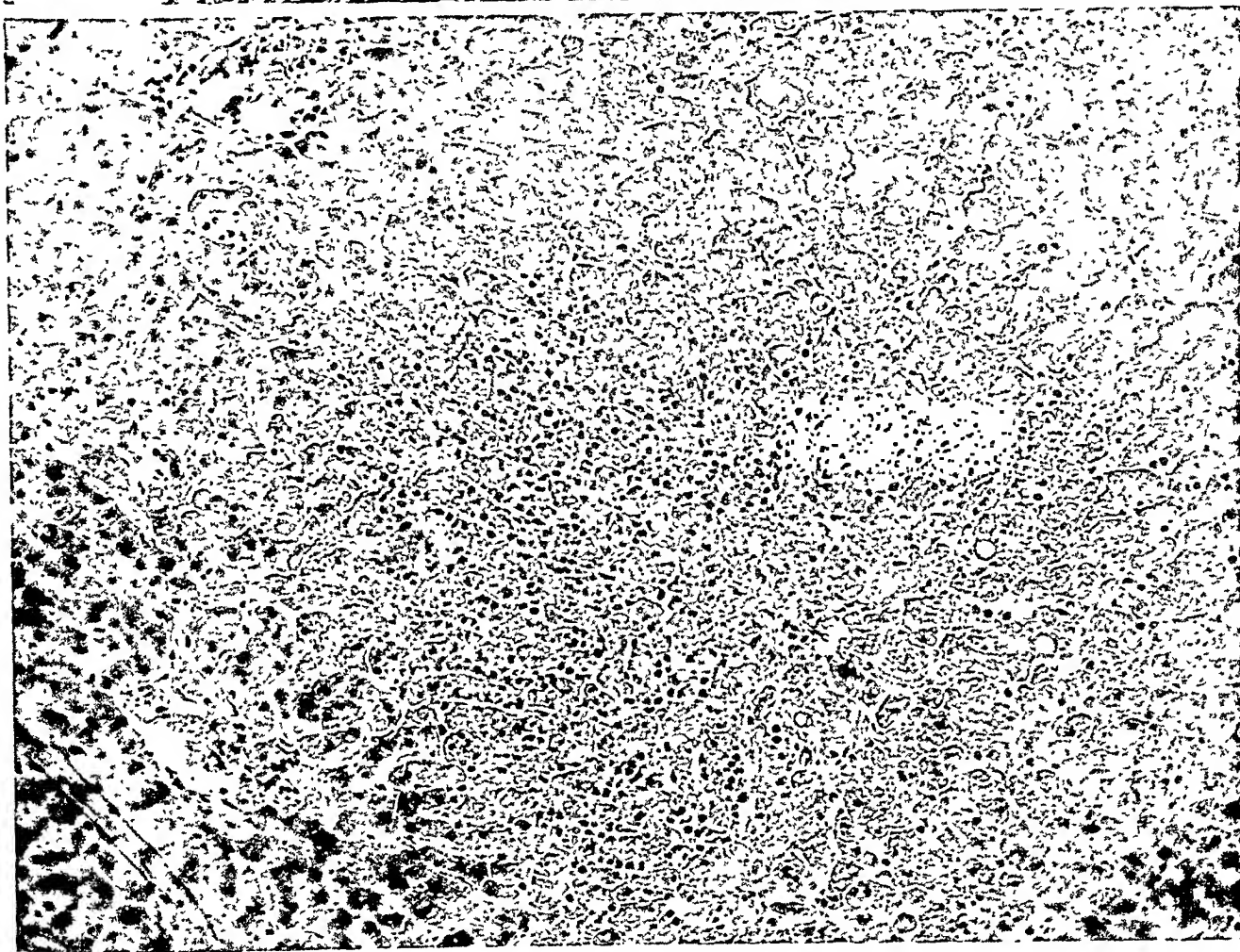
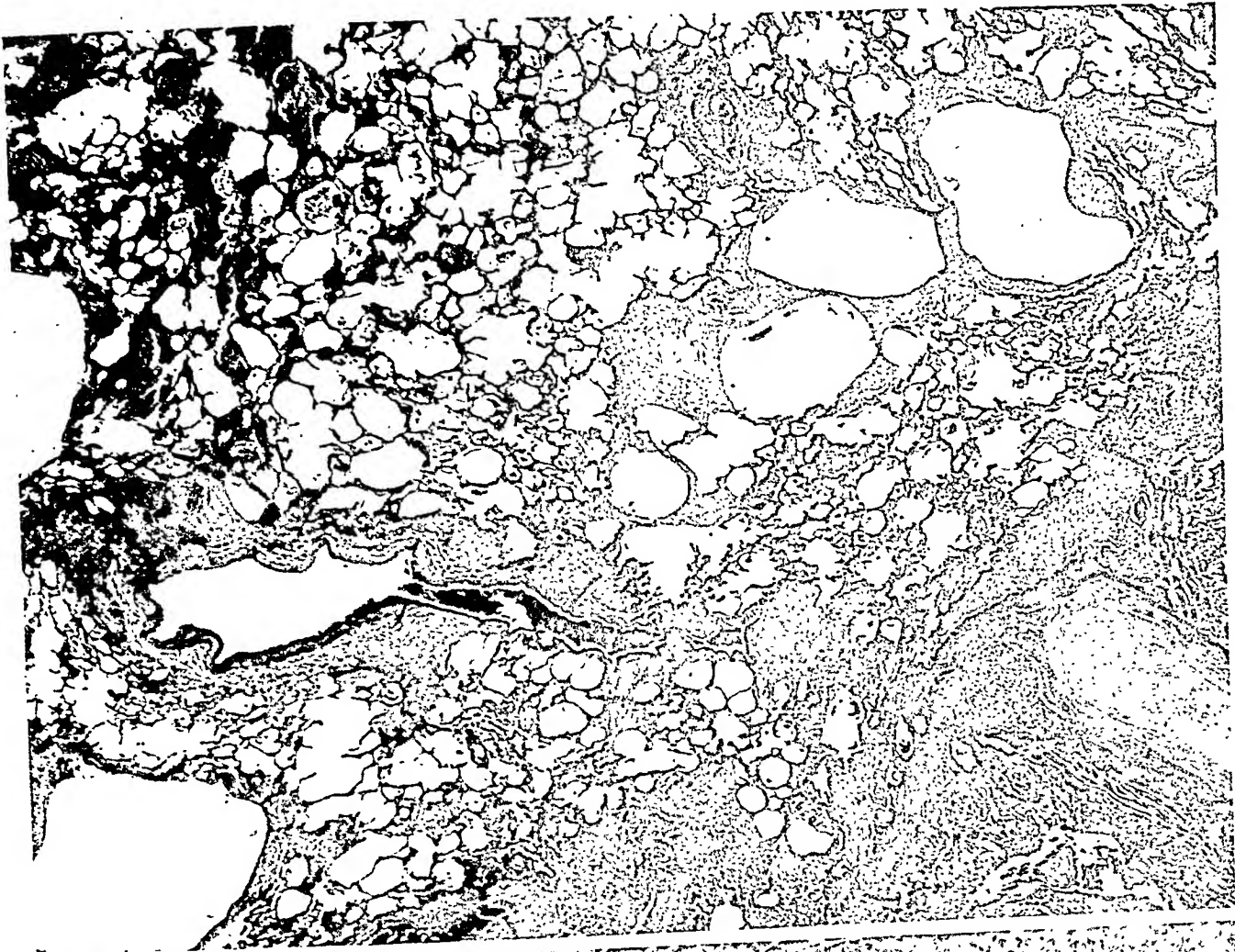


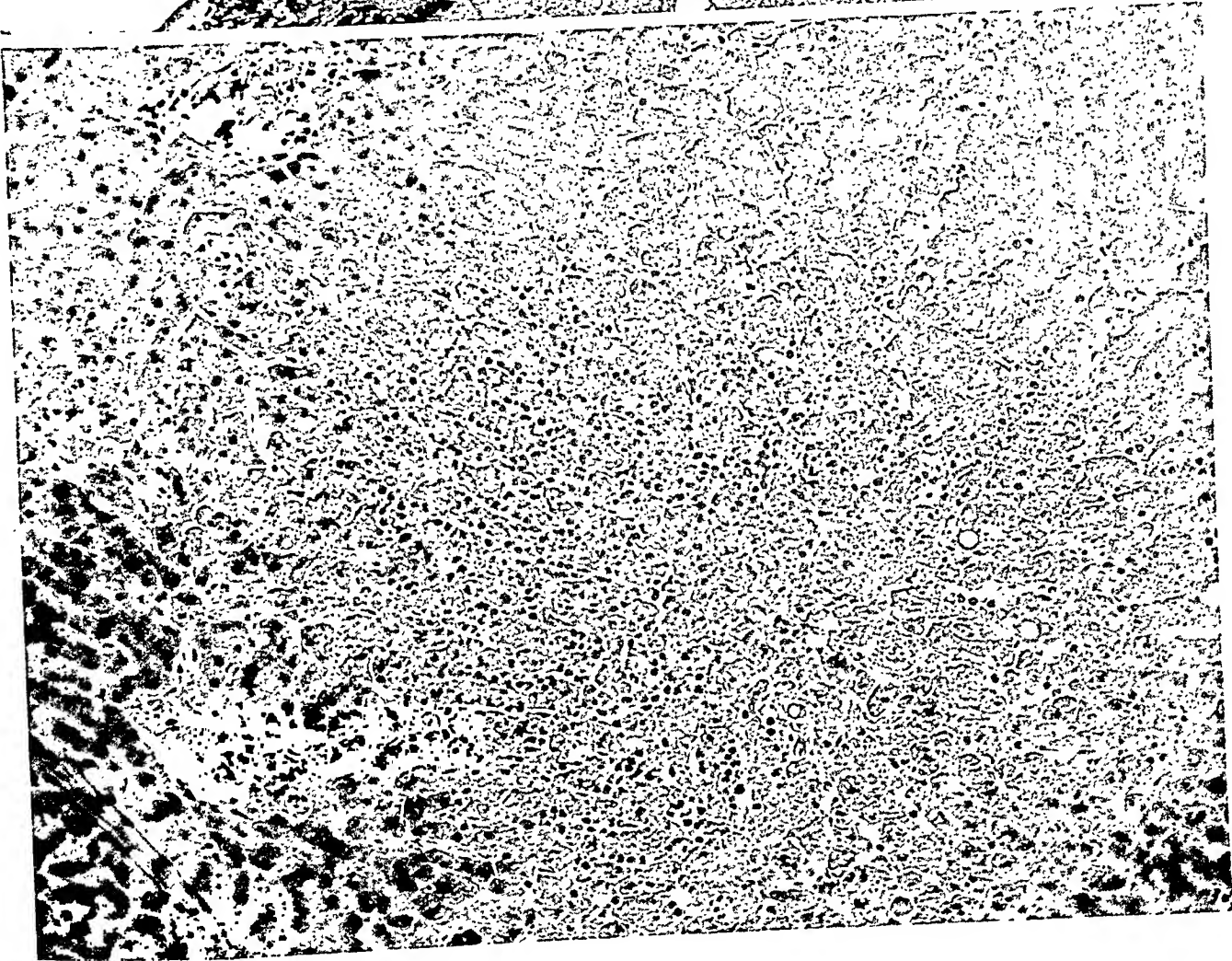
PLATE 191

- FIG. 14. Low-power photomicrograph to show the locations of nodules in the perivascular tissues and in the walls of septa, and the marked emphysema associated with chronic granulomatosis (case C-1). $\times 16$.
- FIG. 15. Liver from a case of acute pneumonitis showing centrilobular necrosis—evidence of systemic toxicity of beryllium (case D-3). $\times 160$.

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PARAGANGLIOMAS

REVIEW OF SUBJECT AND REPORT OF FIVE ORIGINAL CASES *

OSBORNE A. BRINES, M.D., and ELMER R. JENNINGS, M.D.

(From the Department of Pathology, Wayne University, and Receiving Hospital, Detroit, Mich.)

Since Manasse¹ first described an undoubted case of paraganglioma of the suprarenal gland, many interesting facts have come to light regarding these rare tumors. Kohn² described the chromaffin system to which these tumors are related. Suzuki³ first reported a case associated with neurofibromatosis. Labbé, Tinel, and Doumer⁴ described the clinical syndrome of paroxysmal hypertension. However, most of the papers dealing with this subject have been isolated case reports with emphasis on some one phase or another. It is our primary purpose to review the literature of this subject, and on the basis of embryogenesis and histology of the paraganglion cells, to evaluate the previously reported cases and to add 5 cases not previously reported.

ORIGIN AND DISTRIBUTION OF PARAGANGLION CELLS

Hollingsworth⁵ has recently reviewed the literature concerning the development of the sympathetic system. By a process of maturation and differentiation, ganglion cells of the sympathetic system, paraganglion cells, and the sheath cells (Schwann or neurilemma cells) are derived from the cells of the neural crest. The cells destined to become paraganglion cells migrate to the dorsal surface of the sympathetic ganglia to form small, rounded masses in depressions of these ganglia. Because of this close association, the term *paraganglion* has been applied. Similar masses occur in the sympathetic plexuses, the best known of which are the organs of Zuckerkandl which develop along the aorta near the root of the inferior mesenteric artery. Similar collections of these cells have been described in the liver, testes, kidneys, and heart. The paraganglion cells which form the suprarenal medulla are derived from the celiac plexus; in embryos of 7 weeks, masses of these cells grow into the cortical primordium (from mesoderm) and gain a central position.

Several other tissues have been included in the paraganglionic or chromaffin system, prominent among which are the carotid body, the argentaffin cells of the appendix and intestines, and the coccygeal body. The most dispute has concerned the carotid body. Kohn,² studying man and the pig, thought the carotid bodies belonged to the paraganglionic system. He stated that the cells of these tissues are chromaffin cells; that brown granules appear with the use of chromate solutions.

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It is true that the carotid body of pigs contains chromaffin cells, but other workers⁶ do not agree that human carotid bodies have chromaffin cells. The carotid body arises from the mesenchyme of the wall of the third branchial cleft and is closely related to the development of a branch of the glossopharyngeal nerve; thus, its origin is different from that of the paraganglion cells. Smith⁶ has shown that its connections with the cervical sympathetic nerves are made late in the development of the embryo. She stated that chromaffin cells are not constant in the carotid body of man or the rat, but can be regularly demonstrated in those of the pig and cow. Furthermore, neither epinephrine nor any related vasopressor substance has been demonstrated in the carotid body or tumors derived therefrom. Boyd,⁷ in a recent review of the subject, definitely rejected the idea that the carotid body be included in the paraganglionic system. Because of the inconstancy of the presence of chromaffin cells, because epinephrine has never been demonstrated and, most important of all, because of the difference in embryologic development, we will not consider tumors derived from the carotid body as paragangliomas.

There has been less dispute concerning the inclusion of the argentaffin tumors of the appendix and intestine with paragangliomas. Masson,⁸ after studying 50 cases of carcinoid of the appendix, confirmed Huebschmann's⁹ suggestion that argentaffin cells are chromophilic, but pointed out several reasons why these tumors should not be catalogued with the paragangliomas. First, the argentaffin cells are not derived from the neural crest, but are of entodermal origin. Masson wrote: "All the argentaffin cells enclosed in the nerves result from intranervous budding from the glands of Lieberkühn. The budding epithelia then separate from their gland matrix, migrate into the nerves of the mucosa and differentiate." Further, the argentaffin cells often contain lipid substances and these are not seen in paraganglion cells. De Castro¹⁰ has shown that the chromaffin reaction sometimes obtained in the carotid body does not depend upon the presence of epinephrine as the reducing substance, but rather on the action of the lipoids contained in these cells. The same might apply to the lipoids of argentaffin cells and the positive chromaffin reaction.

Lewis and Geschickter¹¹ included argentaffin tumors in their series of paragangliomas, but Geschickter now considers their exact origin to be less certain. For the reasons stated by Masson,⁸ in addition to the fact that no epinephrine or similar substance has ever been demonstrated in these tumors, we prefer not to include argentaffin tumors with paragangliomas.

Some early writers have compared the coccygeal body with the

carotid body and so have included it in the paraganglionic system. Because it has nothing in common embryologically with paraganglion cells, we shall not consider it here.

CHROMAFFIN REACTIONS

Bennett,¹² in describing the suprarenal medulla of the cat, presented an excellent survey of the history and the significance of the chromaffin reaction. Henle¹³ reported that a dark brown color appeared in the suprarenal medullary cells if they had been subjected to the action of chromic acid or potassium dichromate. Following the identification and synthesis of epinephrine shortly after 1900, it became known that the hormone could be easily oxidized and that the oxidized epinephrine was brown. Hartman and Blatz¹⁴ used the chromaffin reaction to indicate the presence of epinephrine in the suprarenal medulla. Ogata and Ogata¹⁵ thought that the brown color was caused by a precipitate formed by the interaction of epinephrine and chromium compounds. For the following decade the chromaffin reaction was used to indicate the amount of epinephrine in the tissues. In 1930 Gerard, Cordier, and Lison¹⁶ showed clearly that the brown color could be produced by mixing epinephrine with strong oxidizing agents other than chromium compounds. This reaction, then, was not really a demonstration of chromaffinity, but a nonspecific reaction between epinephrine (and related compounds) and strong oxidizing reagents such as solutions of potassium iodate. The term chromaffin reaction is apparently a misnomer since the reaction depends on an oxidation reaction and not on chromium affinity. Organic substances which form these complex compounds with oxidizing agents include epinephrine, hydroquinone, resorcinol, aniline, polyphenols, and many others.

Bennett¹² stated that some of these compounds must be present in all tissues of the body and must account for the light brown color seen in all tissues fixed in dichromate. The much darker color of the suprarenal medulla and paraganglia merely indicates a greater concentration of these fuscogenic substances.

Because the chromaffin test is nonspecific, one should not classify a tumor as a paraganglioma simply because of the presence or absence of a positive reaction. Of greater importance are its origin from cells which have been derived from the neural crest and the presence in it of cells capable of secreting a pressor substance. In a practical sense, then, when one is attempting to classify a tumor suspected of being a paraganglioma he should consider the site of origin and the histologic resemblance to paraganglion cells of the suprarenal medulla. The histologic appearance may vary greatly and may be of little help in

classifying and identifying these tumors. We shall consider as being typical paragangliomas only those tumors which arise in the suprarenal medulla, or in association with the sympathetic ganglia, or in the sympathetic plexuses. The discussion will thus be limited and the statistics presented will be at variance with those by authors who have employed the term in a less restricted fashion.

REPORT OF CASES

Case 1

A Negro housewife, 43 years of age, was first admitted to the Detroit Receiving Hospital on December 31, 1945, because of vaginal bleeding and weakness of 5 days' duration. The positive physical findings included pale mucous membranes, soft cervix with patulent os, and a large nodular uterus. Serologic tests for syphilis were negative and the hemoglobin value was 7.0 gm. per 100 cc. of blood. The clinical impression was complete abortion, multiple leiomyomas of the uterus, and secondary anemia. Treatment consisted of pituitary preparations and multiple blood transfusions. She responded well and was discharged on January 11, 1946, to return to the out-patient department. The blood pressure was not elevated. She was re-admitted to the hospital on May 30 in order that definitive treatment for the uterine tumors could be carried out. At this time the blood pressure was 110/70 mm. Hg. The remaining physical findings suggested only multiple leiomyomas of the uterus and secondary anemia. On June 5 supracervical hysterectomy and appendectomy were performed without incident through a midline incision. While exploring the abdomen a retroperitoneal tumor was palpated slightly to the right of the midline and at the level of the third lumbar vertebra. This mass was black, moderately firm, and measured about 6 cm. in maximum diameter. The peritoneum was incised and the mass was found to be attached by a small pedicle to the retroperitoneal tissue. The exact relation to the abdominal aorta was not determined. The postoperative course was uneventful and the patient was discharged on June 17 to the out-patient department. In the succeeding 5 months she was followed at regular intervals and her progress was very satisfactory.

The specimen consisted of a thinly encapsulated, firm neoplasm weighing 30 gm. and measuring 5 by 3 by 2.5 cm. The formalin in which the tumor was immersed was colored brown. Approximately 90 per cent of the tumor was blue-black, there being a small, light-pink area at one end. The demarcation between the pigmented and non-pigmented portions was abrupt. Numerous slit-like cavities were seen in the pigmented portion. The tumor was composed of irregular polygonal cells arranged in solid groups, which were separated one from another by a delicate connective tissue stroma and thin-walled capillaries. In sections prepared from the pigmented areas the neoplastic cells contained a granular pigment, which on low-power examination appeared black and with higher magnification appeared brown-black. Sections from the light areas were almost devoid of pigment. The capillaries, stroma, and alveolar pattern were more prominent in the pigmented areas. The Prussian blue reaction for the presence of

iron was negative. Dopa and chromaffin tests were not done on the fresh material. Staining with the Mallory-Heidenhain technic, as recommended by Gomori,¹⁷ gave negative results. This is an azo-carmin stain with which substances related to epinephrine appear as violet granules in the cytoplasm of chromaffin cells. The pigment was darkened by aqueous silver nitrate and bleached with hydrogen peroxide.

Anatomic Diagnoses. Paraganglioma, benign, retroperitoneal.

Case 2

The patient was a Negro male, 34 years old, who was admitted to Detroit Receiving Hospital because of headaches of several months' duration associated with dizzy spells, nausea, and vomiting. He stated that he had innumerable tumors over his entire body since childhood. His blood pressure was 180/140 mm. Hg. The remainder of the physical examination was negative except for the cutaneous nodules and hypertensive retinopathy. Routine laboratory studies revealed little of note. A calcific mass, apparently within the left kidney, and a similar mass in the right upper abdominal quadrant were demonstrated radiographically. The patient was discharged and re-admitted 7 months later. It was then that the diagnosis of paraganglioma was suspected, for it was learned that laughing or change in his position would precipitate an attack of the previously described symptoms. Confirmatory tests were done, including perirenal insufflation for roentgenographic study. His attacks continued and he died, apparently in shock, before surgical treatment for his disease could be performed.

Multiple, discrete, cutaneous tumors varying from 1 mm. to 4 cm. in average diameter were distributed universally. There was an equinovarus deformity of the left foot. Examination of the contents of the cranial cavity revealed mild fibrosis of the leptomeninges. In the right cerebral hemisphere there was an elongated softening of the white matter. There was also a small hemorrhage in the pineal body. The heart weighed 425 gm. with a ventricular ratio of 3.0 (normal, 1.6 to 2.0). The peritoneal cavity contained 500 cc. of clotted and unclotted, recently shed blood. Beneath the right leaf of the diaphragm there was a hematoma, partially organized, measuring 8 cm. in maximum dimension. Within the spleen there was a subcapsular hematoma 4.5 cm. in diameter. In the walls of the esophagus, stomach, duodenum, small intestine, and large intestine were numerous firm, yellow-white tumors varying in size from 2 mm. to 2 cm. In the medial pole of the right suprarenal gland there was an encapsulated, spherical tumor, 3.5 cm. in diameter, which was reddish brown and hemorrhagic, and weighed 19 gm.; the uninvolved portion of the suprarenal gland weighed 5 gm. The opposite suprarenal body was diffusely enlarged, measuring 7 by 3 by 1 cm. and weighing 16 gm. Serial sections revealed no nodular lesion. Between this suprarenal body and the

superior pole of the kidney there was an area of recent hemorrhage measuring 4 cm. in maximum dimension.

In the brain there were atherosclerosis, arteriolar sclerosis, and thrombosis associated with multiple areas of softening and glial proliferation. The subcutaneous tumors and those throughout the gastro-intestinal tract were composed of encapsulated masses of interlacing bundles of fibrillar connective tissue with elongated nuclei, and no definite nerve bundles were seen in the sections examined. The lungs showed diffuse pulmonary edema, purulent bronchitis, passive congestion, and confluent lobular pneumonia. Nothing remarkable was noted in the sections prepared from the gastro-intestinal tract save for the previously described neurofibromas. In the kidneys, definite arteriolar sclerosis was seen which was indistinguishable from that frequently associated with essential hypertension. In addition a recent intracapsular hemorrhage was noted.

The suprarenal tumor was composed of polygonal cells measuring about 35 μ in maximum dimension which contained large vesicular nuclei with one prominent nucleolus per cell. The cytoplasm contained very fine, densely packed granules which were stained purple with hematoxylin and very closely resembled those of the secretory cells of the normal suprarenal medulla. These cells were arranged in cords and nests which were surrounded by a delicate connective tissue stroma. The tumor was highly vascular, containing many thin-walled capillaries and sinusoidal spaces, often markedly dilated. Some of the tumor cells were smaller (15 μ), more pink, and had dense nuclei without nucleoli. A portion of the tumor was placed in Orth's solution in order to demonstrate possible chromaffinity of the cells. When stained with Schmorl's technic¹⁸ the nuclei appeared deep blue-green while the cytoplasm of the tumor cells contained numerous, very fine, yellowish green granules. Confirmation of this positive chromaffin reaction was obtained by staining formalin-fixed tissue with Heidenhain's azocarmine according to Gomori's suggestion.¹⁷ With this technic, the granules were violet, corresponding to the granules of the suprarenal medulla which was used as a control. An interesting incidental feature was that the nucleoli of the tumor cells stained brilliant red.

Anatomic Diagnoses. Paraganglioma, benign, right suprarenal gland; neurofibromatosis, skin and gastro-intestinal tract; heart disease, hypertensive, with left ventricular hypertrophy; pneumonia, lobular, bilateral; hemoperitoneum; hematoma, subcapsular, spleen, kidney; arteriolar sclerosis, generalized; encephalomalacia; talipes equinovarus, left; kyphoscoliosis, thoracolumbar spine.

Case 3

The patient was a Negro male, 33 years of age, who was admitted to the Medical Service of the Detroit Receiving Hospital with a chief complaint of constipation, gas on the stomach, nausea, vomiting, and hiccoughs of 3 days' duration. He had had epigastric pain, relieved by soda and milk, for 10 years. The vomitus had been bloody on two occasions during the week prior to admission. He had had malaria as a boy and there was an indefinite history of syphilitic chancre 11 years before admission. Physical examination revealed slight icterus, decreased expansion of the right chest, and flatness. There was no cardiac hypertrophy; the abdomen displayed only slight tenderness, some distention, and spasticity of the rectus muscle on the right side. No abdominal masses were palpated. Results of routine examination of the blood and urine were normal. The working diagnosis was cholecystitis with cholelithiasis, and the patient was treated conservatively. During hospitalization, he became progressively worse and expired 5 days after admission.

The principal positive findings at necropsy were in the abdominal cavity. Generalized peritonitis had resulted from perforation of two ulcers of the duodenum and stomach. In the left suprarenal gland there was a neoplastic nodule measuring 2.5 cm. in diameter, confined to the gland. The remaining viscera appeared normal.

The neoplasm was composed of cells which varied greatly in size from 10 to 30 μ and were arranged in poorly formed cords and nests. The cells were roughly elliptical in most areas, but spherical in others. The cell boundaries were indistinct. The nuclei of the tumor cells, for the most part, contained moderately dense, diffusely distributed chromatin. However, the nuclei varied greatly in size and shape and contained no nucleoli. Some of the cells with indistinct outlines appeared to be giant cells and contained from two to five nuclei. The cytoplasm of the tumor cells was abundant and contained many fine, densely packed, violet-staining granules. Some of the tumor cells had undergone hydropic change. The stroma of the tumor, for the most part, was delicate, fibrous, and sparse. However, in some areas there was a very dense fibrous stroma. There were many dilated, thin-walled capillaries throughout the tumor. There was a moderate amount of necrosis and hemorrhage. At one edge of the section a rim of compressed suprarenal cortical tissue was seen.

Anatomic Diagnoses. Ulcers, duodenal and gastric, with perforation, generalized peritonitis, and abscess formation; paraganglioma, benign, left suprarenal gland.

Case 4

A white female, 21 years old, was first admitted to the Detroit Receiving Hospital on December 6, 1937. She had known that she had high blood pressure for 6 years prior to admission. This hypertension had been discovered in 1931 as part of a general physical examination because of symptoms of nervousness, palpitation, and headache. During the succeeding years the symptoms became progressively worse

and were accompanied by nervousness and irritability. In 1934 a partial thyroidectomy was performed at another hospital, at which time there was moderate elevation of the basal metabolic rate. Following this operation her symptoms were somewhat relieved but later returned, and in 1937 she was hospitalized for a "nervous breakdown." At this time her basal metabolic rate was plus 72 and another thyroid operation was performed. She felt better for a short time, but her symptoms returned and she became progressively worse. At the time of admission to the Detroit Receiving Hospital she was complaining of palpitation, headache, flushed face, and nervousness. Physical examination revealed only minimal changes of the vessels in the optic fundus. A remnant of the thyroid gland was palpable. The heart was moderately enlarged to the left with a systolic blowing murmur over the precordium and an accentuated aortic second sound. Blood pressure at the time of admission was 194/132 mm. Hg. Repeated determinations of the blood pressure revealed that the systolic pressure varied from 150 to 240 and the diastolic from 100 to 150. Routine studies of the blood and urine were normal. The Kline test was negative. The basal metabolic rate was plus 16. Blood cholesterol was 192 mg. per 100 cc. of plasma. An electrocardiogram indicated sinus tachycardia with no definite evidence of myocardial involvement. An excretory urogram revealed a slight outward displacement of the right kidney, and in the position of the left suprarenal gland a definite area of calcification was evident. While on the ward her condition remained unchanged. Marked variations in blood pressure determinations were noted. On January 18, 1938, an exploratory operation of the left suprarenal area was performed and a retroperitoneal tumor was found, situated superiorly and mesially to the upper pole of the left kidney and extending posteriorly along the spinal column at approximately the level of the 12th thoracic vertebra. The anterior surface was covered by a complicated mass of veins of considerable size, some being 2 mm. in diameter. Intravenous fluids, including whole blood, were administered. The blood pressure fluctuated greatly; at one time the systolic pressure was 290 and a reading was unobtainable at another time. All of the tumor was removed except the extreme posterior portion lateral to the spine. At this point in the operation the patient stopped breathing, and quantities of rust-colored, frothy fluid exuded from the nostrils and throat. Despite supportive measures, she expired. Permission for necropsy was not granted.

The specimen was submitted in several pieces, having an aggregate weight of 20 gm. They were irregular, dark brown, and soft. The formalin in which the tissue had been immersed for 24 hours was discolored brown. The tumor cells were large, irregular, and contained hyperchromatic nuclei with abundant deep-staining cytoplasm. Many of the cells were vacuolated. There was a considerable variation in nuclear size and staining intensity. A few mitotic figures were seen. Some of the cells were multinucleated, containing two to four nuclei. Thick and thin-walled veins were present throughout the tumor. There was an incomplete, thick, fibrous capsule. A portion of the tumor was stained with Giemsa's stain and no olive-green granules were seen. This, then, would be a negative chromaffin reaction with the Schmorl method.¹⁸ However, when the tissue was stained with azocarmine, there was a definite positive reaction, the cytoplasm of the tumor cells

containing gray-blue, coarse granules. The nuclei were red except the large vesicular ones which had prominent red nucleoli.

Anatomic Diagnosis. Paraganglioma, benign, retroperitoneal.

Case 5*

The patient was a white woman, 50 years of age, who had suffered from dyspepsia for many years. During the past 2 years she developed a chronic, persistent hypertension with blood pressure of approximately 160/90 mm. Hg. There was a gradual onset of exertional dyspnea and ankle edema which were relieved by the administration of digitalis. Four days prior to hospitalization she was seized by an attack of nausea, vomiting, and severe pain in the right upper quadrant. At the time of admission to Woman's Hospital her temperature was 100.2°F.; pulse rate, 90 per minute; and blood pressure, 190/110 mm. Hg. There was tenderness on the right side of the abdomen associated with a poorly defined mass. The results of hematologic and urine studies were within normal limits. On November 21, 1936, 6 days after admission, a cholecystectomy was done. During the operation, the blood pressure fluctuated widely. The gallbladder was thickened and contained two large stones and a large amount of thick purulent material. Her postoperative course was satisfactory during the first 3 days. She then became apprehensive, nauseated, and vomited several times. Temperature, pulse rate, and respirations increased and she became comatose. She died 9 hours after the onset of these symptoms.

There was a fairly well developed growth of hair on the upper lip and chin, but no other signs of masculinization. The heart weighed 300 gm., but was otherwise normal. There was hemorrhagic edema of the lungs. The right suprarenal gland was diffusely enlarged and weighed 25 gm. The left suprarenal gland was replaced by a firm, encapsulated tumor which weighed 600 gm. and measured 11 by 10 by 8 cm. It was easily removed from the surrounding structures. On cut section, it was seen to be highly vascular, soft, and white in some areas and pink in others. The epinephrine content was ascertained according to the method of Folin, Cannon, and Denis¹⁹ and it was found that 10.2 gm. of the tumor contained 24.6 mg. of epinephrine. This would indicate that the entire tumor contained about 1400 mg. of epinephrine.

In the sections prepared from paraffin-embedded tissue and stained with hematoxylin and eosin, the tumor cells were seen to be polyhedral with indistinct cell boundaries. The average size of the cell was 35 μ . The nuclei in most cases were large, vesicular, and nearly all of them had prominent nucleoli. Some cells appeared to be binucleated. The cytoplasm contained fine, violet-staining granules. The cells were arranged in cords and nests and were separated from one another by delicate connective tissue stroma with a rich capillary network. Some

* Reported with the permission of Drs. Earl G. Krug and D. C. Beaver.

of the capillaries were distended and contained very many white blood cells. There was a remarkable resemblance between the microscopic pattern of the tumor and the normal suprarenal medulla. There was no necrosis or hemorrhage. The capsule was incomplete. Many thin- and thick-walled veins were present. A portion of the tumor was fixed in a chromate solution and sections examined from this material revealed brown granules in the cytoplasm of the neoplastic cells.

Anatomic Diagnoses. Paraganglioma, benign, left suprarenal gland; hypertrophy, myocardial, mild; edema, hemorrhagic, lungs; gallbladder, absence of, acquired.

REVIEW OF THE LITERATURE

The three terms commonly used in the literature by which these tumors are designated are paraganglioma, pheochromocytoma, and chromaffinoma. For reasons previously stated, chromaffinoma seems objectionable. Pick,²⁰ in 1912, suggested that the term pheochromocytoma (tumor of dark-colored cells) be used for those tumors arising in the suprarenal medulla and that the extra-suprarenal tumors be called paragangliomas. Some authors have followed his suggestion, but since this separation is arbitrary and does not reflect a fundamental distinction, we prefer to designate all of these tumors as paragangliomas. This term seems most acceptable since it refers to the cells from which these tumors are derived.

Many excellent reviews of the literature have appeared during the past 15 years. Belt and Powell²¹ collected all of the cases reported up to 1934. In 1941 Biskind, Meyer, and Beadner²² contributed a compilation of the cases treated surgically which had been reported prior to that time. Rosenthal and Willis²³ reviewed all of the cases of paraganglioma associated with neurofibromatosis. Green²⁴ studied the incidence of chronic hypertension and analyzed 50 reported cases. McGavack, Benjamin, Speer, and Klotz²⁵ analyzed the reports of malignant paraganglioma. Mackeith,²⁶ in his article submitted for publication in May, 1943, stated that 165 of these tumors had been described. Since that time approximately 40 more cases have been added. The 5 cases described in this paper bring the total number of reported cases to about 210.

These tumors occur with equal frequency in the male and the female, chiefly during young adult life, and usually between the ages of 20 and 50 years. However, the age variability is great: one of Fingerland's²⁷ cases was that of a 71-year-old male, and Soffer, Mencher, and Colp²⁸ reported an active tumor occurring in a 7-year-old girl. The few reports of cases occurring in infants and very young children are

all highly atypical. The 1-year-old infant described by Neff, Tice, Walker, and Okerblad²⁹ displayed hirsutism, precocious development of the genital organs, and obesity. The tumor which occurred in the mediastinum of a 4-year-old boy, reported by Wahl and Robinson,³⁰ contained neuroblastic elements with widespread metastases and thus does not belong in the category of paragangliomas.

About 90 per cent of these neoplasms have occurred in the suprarenal glands and there is a slight, but definite, predilection for the right gland. All of the extra-suprarenal tumors have occurred in the abdominal cavity or retroperitoneal area except two. One of the two acceptable intrathoracic paragangliomas was reported by Philips³¹ from the apex of the pleural cavity, apparently arising from the first left thoracic ganglion. The second was reported by Miller³² as occurring in a paravertebral position in the right pleural cavity opposite the 6th interspace. Of those described as extra-suprarenal but intra-abdominal, 10 have occurred in the organ of Zuckerkandl, 3 in the retroperitoneal area (including our first case), one at the hilus of a kidney, and one at the celiac ganglion. Many others have been reported in the carotid body, the appendix, and the sacrococcygeal body, but these tumors are not included in this review. Waaler³³ has collected all of the extra-suprarenal paragangliomas reported up to 1945.

These tumors are multiple in about 10 per cent of the cases. In 18 reported cases they have occurred simultaneously in both suprarenal glands. The importance of this fact is emphasized in the report of Knake³⁴ in which is described an 11-year-old boy who had one suprarenal tumor removed but died 5 weeks later. A similar benign tumor was found in the opposite suprarenal gland at necropsy. In addition to those with bilateral tumors, other reports describe apparently benign but multiple tumors. One of Fingerland's²⁷ cases had a tumor of the right suprarenal gland and the organ of Zuckerkandl. The important clinical implications of these facts must be apparent.

The tumors have varied in weight from 5 gm. to more than 2 kg. One tumor described by Soffer *et al.*²⁸ weighed 2 kg. after much blood had been removed. Typically, the tumors are encapsulated, spherical or oval, and reddish brown. Many are hemorrhagic and necrotic, and have undergone cystic degeneration. Often a rim of suprarenal cortex can be seen at one edge of the specimen. In our case 1 the tumor was blue-black because of the high melanin content, but this is unusual.

In the tumors which develop the more typical and characteristic histologic pattern there is an unmistakable resemblance to the suprarenal medulla. The nuclei of the tumor cells are large, vesicular, and frequently have prominent nucleoli. The cytoplasm is abundant, mak-

ing each cell polygonal. Often fine granules can be seen in the cytoplasm, even in sections prepared from formalin-fixed tissues and stained with hematoxylin and eosin. Frequently the cells are arranged in cords and nests and are separated by a delicate connective tissue stroma. Usually, there is an abundant capillary network. When fixed in Orth's solution and stained by the Schmorl technic¹⁸ the nuclei are blue and the cytoplasm often contains olive-green granules. When fixed in formalin and stained with azocarmine, as suggested by Gomori,¹⁷ the nucleus usually is pale, the nucleolus a brilliant red, and the cytoplasm often contains bluish violet granules. However, many of the tumors are atypical and may pose a problem in histologic diagnosis. Some tumors are composed of small cells with dark, pyknotic nuclei and no demonstrable nucleoli. The cells may be fusiform or spindle-shaped. Great variation in size and shape may be present, including the formation of giant cells. The cytoplasm may be scanty and contain no granules. The reactions to the Schmorl¹⁸ and azocarmine stains may be negative. The cells may contain a large amount of melanin. Ganglion cells may be present. The pathologist must then give special consideration to the clinical features of the case.

It is difficult to ascertain the real incidence of hormonal activity of these tumors. Before 1922, at which time the relationship of these tumors to paroxysmal hypertension was first clearly described,⁴ most of the reports consisted of necropsy findings and about one-half of the tumors apparently were inactive. During the past 20 years many clinical as well as pathologic diagnoses have been made and, quite naturally, the percentage of hormone-producing tumors has increased. During the past 4 years about 30 cases have been reported, all characterized by symptoms of epinephrine production. It is interesting that of the 9 tumors associated with generalized neurofibromas, only 2 (including our case 2) have produced the suprarenal-sympathetic syndrome.

There is some confusion in the literature concerning the frequency with which these tumors are malignant. McGavack *et al.*²⁵ stated that only 8 cases of definitely malignant paraganglioma had been reported and that all of these were physiologically inactive, *i.e.*, did not produce the suprarenal-sympathetic syndrome. The following year MacKeith²⁶ stated that 17 malignant paragangliomas had been described and that some of them had been active. The reason for this disagreement is that MacKeith apparently accepted all of the cases reported as malignant, whereas McGavack critically analyzed all of the reported cases and accepted only about half of them. McGavack categorically stated that it is not possible to separate the malignant from the benign

tumors histologically, and that "all of the cases in which metastases did not occur should be classified as benign." On this basis, he accepted only 7 cases and added another. We would insist upon invasion in lieu of metastasis as a criterion of malignancy. We believe that Fein and Carman's³⁵ case is a typical example of a benign paraganglioma incorrectly diagnosed. This patient was a 27-year-old female; at necropsy an encapsulated tumor of the right suprarenal gland was found which was assayed and found to contain epinephrine. There was no cachexia and no local invasion by the tumor or metastasis. Furthermore, the photomicrographs revealed a pattern which is compatible with that of a benign paraganglioma although the diagnosis given is medullary carcinoma of the suprarenal gland (malignant pheochromocytoma). Many similar examples could be cited to account for the disparity between the figures of Mackeith and McGavack. Since McGavack's report, no acceptable malignant paraganglioma has been reported. The presence of multiple tumors does not constitute proof of malignancy. There have been 18 reported cases of bilateral paragangliomas. McGavack has accepted as malignant only 5 cases with bilateral tumors. The bilateral paragangliomas of the suprarenal gland reported by Knake³⁴ were clearly benign. Fingerland's²⁷ case in which tumors were found in the right suprarenal gland and the organ of Zuckerkandl was reported as one of benign neoplasm.

In summary, it can be stated that a paraganglioma which is locally invasive or has metastasized should be considered malignant. If there are multiple tumors and one or more of the nodules is located where paraganglion cells are never found normally (*e.g.*, lymph node or bone), it is a malignant neoplasm. If the tumor is well encapsulated but the cells appear to be anaplastic, the observer must be cautious in classifying it as malignant. If the tumors are multiple and all of them are situated where paraganglion cells are normally found (*e.g.*, both suprarenal glands, one suprarenal gland and the organ of Zuckerkandl), again one must be cautious in interpreting one tumor as being primary and the others metastatic.

For the 8 cases of malignant paraganglioma accepted by McGavack *et al.*,²⁵ the following features were observed: All occurred in the suprarenal gland; none was associated with paroxysmal hypertension or the suprarenal-sympathetic syndrome; the age range was from 30 to 68 years with an average of 45.5 years; 5 of the 8 were bilateral; the outstanding clinical features were loss of weight, cachexia, and pain at the primary and metastatic tumor sites. In McGavack's own case, the picture of cachexia was so extreme that diagnoses of Addison's and Simmond's diseases were considered. In 3 of

the 8, a palpable mass was present. The regional lymph nodes were involved in all. Other metastatic sites were as follows: Thoracic lymph nodes, 5; liver, 4; bones, 3; lungs, 3; pleura, 2; skin, 2; intestines, 1; kidney, 1.

In several of the reported cases increased pigmentation of the skin has been described. There are two possible interpretations of this observation. The more obvious is that the expanding tumor has produced pressure on the suprarenal cortex and that the increased pigmentation is a result of cortical deficiency. There is abundant evidence, both clinical and experimental, that deficiency of suprarenal cortical hormones causes deposition of melanin in the skin. At least 4 cases of paraganglioma have been reported in which clinical features of Addison's disease were present.²⁵ In 2 there was excessive loss of sodium chloride in the urine and a retention of potassium. But the disturbing fact is that in none of these cases could encroachment on the suprarenal cortex be demonstrated. In McGavack's²⁵ case, for example, there was stretching of the cortex on the side of the tumor, but the opposite suprarenal gland was uninvolved, and serial sections of all endocrine glands revealed no significant change. The cause for the development of Addison's syndrome remains obscure. The other possible interpretation of the increased skin pigmentation is that it is related to neurofibromatosis, it being well known that areas of deep pigmentation often precede the development of neurofibromas.

Before concluding a discussion of the relationship of paragangliomas to disturbed suprarenal cortical metabolism, mention should be made of LeCompte's³⁶ case of a 31-year-old white female who displayed the signs and symptoms of the androgenital syndrome. Since this syndrome is sometimes due to hyperfunction of the suprarenal cortex, it might be thought that stretching of the suprarenal cortex stimulated it to activity, an effect exactly the opposite of that suggested in the preceding paragraph to explain the production of Addison's syndrome.

Many causes and mechanisms of death have been described for patients with paragangliomas. Of those with malignant tumors the cause of death was attributed to the cancer. Patients with tumors which were nonfunctional and apparently produced no epinephrine died of unrelated causes. When the epinephrine-sympathetic syndrome was present, the cause of death was usually attributed to some effect which the tumor produced. Very often the anatomic changes at death are minimal and the possibility of acute epinephrine intoxication arises. Dolgin³⁷ reviewed the causes of death in some detail and cited the work of Raab³⁸ on the toxic effects of epinephrine-like substances.

EFFECTS OF EPINEPHRINE

One of the most interesting aspects of these tumors, and certainly the one most widely discussed in the literature, is the physiologic change produced in the patient as the result of the production of an epinephrine-like substance. In this respect paragangliomas resemble the active islet cell tumors of the pancreas, the virilizing tumors of the ovaries and suprarenal cortex, the basophilic tumors of the pituitary body, and others. The first clear-cut description of the syndrome of paroxysmal hypertension was written in 1922 by Labbé, Tinel, and Doumer.⁴ Following this report there was rapid advancement in knowledge of the activity of these tumors. For several reasons it was natural for the early workers to suspect that they produced a substance similar to epinephrine. The frequent occurrence of these tumors in the suprarenal gland, the histologic similarity to the suprarenal medulla, the chromaffin reaction, and the striking resemblance of the spontaneous attacks of headache, hypertension, sweating, and pallor to the known physiologic effects of epinephrine all pointed to production by the tumor cells of a substance similar to epinephrine. That a vasopressor substance is present in an active paraganglioma can be demonstrated by several methods. The one most commonly used has been the injection of a neutralized acid extract of the tumor into a lightly anesthetized dog and a comparison of the pressor effect produced with the action of a known quantity of epinephrine. Hyman and Mencher³⁹ prepared a solution from a patient's blood while she was having an attack of paroxysmal hypertension. By perfusion of this material through a rabbit's ear, they were able to demonstrate a pressor effect. That this substance was similar to epinephrine was further supported since the effect could be reversed by ergotamine. In several cases an elevated level of the blood potassium has been reported. The significance of this is apparent when one considers that the injection of epinephrine into a human being causes an elevation of blood potassium of as much as 87 per cent above normal.²⁶ Epinephrine in a crystalline form was obtained from a tumor by Kelly, Piper, Wilder, and Walters⁴⁰ in 1936. DeVries, Mandl, Rachmilevitz, and Ungar⁴¹ used a colorimetric method (Ewins'⁴² test in a buffered solution) in estimating the epinephrine content of the tumor to be studied. Mortell and Whittle⁴³ injected dogs intravenously with diluted fluid obtained from a cystic tumor which they encountered, and made direct measurements of the vasopressor effect. Furthermore, they cited Strömbeck and Hedberg's⁴⁴ description of a chemical method based on the decolorization of methylene blue. The latter had described a case of which, during the

paroxysms of hypertension, the blood contained one thousand times the normal amount of epinephrine and during the remissions showed an epinephrine content which was elevated thirty-fold. It is true that these methods are often indirect and leave much to be desired, but certainly they prove that epinephrine (or a substance closely allied to it) is often produced by these tumors.

It is usually stated that bio-assay of the tumor must be done shortly after it has been removed. However, nowhere have we found descriptions of decreasing vasopressor activity as the time interval between removal and determination of epinephrine increased. In Mortell and Whittle's case⁴³ there was no decreased potency at the end of 48 hours, when the tumor was stored in the ice box. Fingerland²⁷ reported 2 cases in which he tested the formalin, alcohol, and Bouin's solution in tumors which had been preserved for 6 years, and demonstrated a strong epinephrine reaction. Since epinephrine is readily destroyed by oxidation in alkaline solution, one should store material to be assayed in an acid solution. The amount of pressor substance contained in paragangliomas has been reported to vary from 6.7 to 2300 mg.⁴⁵ From the normal human adult suprarenal glands only about 8 mg. are recoverable.

Varied clinical effects may be produced by these tumors. The syndrome first described and the one most often seen and recognized is that of paroxysmal hypertension. More recently, excellent reports have been made of cases simulating chronic essential hypertension,^{24, 46} diabetes mellitus,⁴⁷ hyperthyroidism,^{45, 48} and adynamic ileus.⁴⁹ Some patients have exhibited signs and symptoms of several of these categories. Rodin⁵⁰ has described in great detail the ophthalmologic changes which occur with this disease. Mackeith²⁰ has discussed the electrocardiographic changes. In Mortell and Whittle's⁴³ case, electroencephalograms were taken during the paroxysms and were found not to be altered. Hyman and Mencher³⁹ measured temperatures of the peripheral skin and found them to be lowered, presumably because of vasoconstriction. In the 2 cases reported by McCullagh and Engel⁴⁸ an elevated urea clearance was demonstrated, which suggests increased renal blood flow; in one there was also severe polyuria, arousing a suspicion of diabetes insipidus.

Several tests have been devised which have been useful for studying a patient suspected of having a paraganglioma. All of them are similar in that they are attempts to provoke an attack. One of the first was suggested by Coller, Field, and Durant⁵¹ and consisted of a subcutaneous injection of a minute amount of epinephrine. Roth and Kvale⁵² have induced attacks with the use of histamine. The mechanism is thought to depend upon the fact that histamine is antagonistic

to epinephrine and causes a temporary reduction in the circulating amount of that substance. However, the effect is temporary and thus the tumor responds with a "rebound" liberation of a large amount of epinephrine. Among the other methods might be mentioned the immersion of the feet in cold water (cold pressor test), the injection of insulin, and hyperventilation (which is thought to cause the diaphragm to press on the tumor). All of these tests have been successful in some cases and unsuccessful in others, so that there is little to recommend one rather than another.

Perirenal air insufflation preparatory to radiographic examination has been advocated by Hyman and Mencher³⁹ and has been very useful in their hands. Others have hesitated to employ this procedure because patients with these tumors are unstable and disastrous results have ensued. It may be that the attempted perirenal insufflation for the patient of our case 2 had something to do with his demise. Scout films of the abdomen have been helpful in many cases, for calcification in the kidney region has been seen. Often these shadows have been misinterpreted as being tuberculous in origin.

RELATION TO NEUROFIBROMAS

In the second case of our series, an active paraganglioma of the right adrenal was associated with neurofibromatosis of skin and viscera. Suzuki³ was the first to report the simultaneous presence of these two neoplastic diseases, and the literature now contains 8 previously reported cases. In only one of these was the paraganglioma active, producing the suprarenal-sympathetic syndrome.⁵³ Our case 2, then, is the second to be reported in which a paraganglioma, multiple neurofibromas, and paroxysmal hypertension have occurred together.

The co-existence of these two neoplastic conditions in a frequency of 1 to 23 suggests that the relationship is more than fortuitous. Nearly all authors reporting such cases have made this comment, but nowhere have we found a satisfactory explanation of this concurrence. As has been described above, the paraganglion cells are derived from the migrating undifferentiated cells of the neural crest. Harrison⁵⁴ has demonstrated experimentally that when the neural crest is divided from the neural tube, the motor nerves continue to develop, but are devoid of the normal sheaths (Schwann cells). This indicates that the sheath cells, like the paraganglion cells, are derived from the neural crest. Stout⁵⁵ recently reviewed the histogenesis of neurofibromas and stated that Schwann cells play the dominant rôle in the formation of these tumors. These accumulated facts suggest, then, that paragangliomas and neurofibromas are both derived from cells which have ultimately come from the neural crest. Several writers have reported

cases demonstrating tumors consisting of combinations of ganglioneuromas, neuroblastomas, and paragangliomas.^{30, 56, 57} These have been accepted as being expressions of different degrees and directions of differentiation from common primordial cells. An extension of this concept might explain the concomitant occurrence of neurofibromas and paragangliomas.

MELANIN PRODUCTION

One of the cases reported by us (case 1) is a retroperitoneal paraganglioma which was deeply pigmented. In attempting to identify the pigment, four substances were considered: hemosiderin, lipochrome, chromaffin granules, and melanin. The first three were eliminated from consideration and the pigment interpreted as melanin because it was iron-free, not soluble in fat solvents, bleached with hydrogen peroxide, and darkened with 5 per cent aqueous silver nitrate. Baker⁵⁸ has shown that these are the only chemical reactions which are of much practical value in the identification of brown pigment.

Tyrosine, when oxidized in the presence of the enzyme tyrosinase, yields melanin.⁵⁹ It can also be used as the precursor of epinephrine.⁶⁰ The possibility of a similar source for melanin and epinephrine and the close chemical relationship of these substances make it seem reasonable that a neoplasm possessing the potential capacity to produce epinephrine might also produce melanin.

Millar⁶¹ has reported a case of malignant ganglioneuroma of the 7th thoracic sympathetic ganglion in which the ganglion cells contained melanin. Masson⁶² has traced the origin of melanomas of the skin to Meissner's corpuscles which are composed of modified Schwann cells. Hyperpigmentation of the skin occurs before the development of, and in association with, multiple neurofibromas. Thus it would appear that a tumor derived from the cells which have origin from the neuroectodermal cells of the neural crest (paraganglioma, ganglioneuroma, neurofibroma, and ordinary melanomas of the skin) is apt to produce melanin. We are well aware that the definitive histogenesis of all of these tumors is not finally settled, but we believe that the accumulating facts lend support to the opinion of a common origin for these diverse neoplasms.

SUMMARY AND CONCLUSIONS

On the basis of the origin and distribution of paraganglion cells, tumors arising in the carotid body, appendix, and coccygeal body have been excluded from the group of paragangliomas. The chromaffin reactions are not specific for paragangliomas.

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The previously reported cases of paraganglioma, reviewed with regard to age and sex distribution, location of tumors, hormonal activity, and histologic appearance, exhibit a variability of cytologic pattern and a marked degree of pleomorphism, which is, nevertheless, consistent with a diagnosis of benign paraganglioma. A diagnosis of malignancy should not be made in the absence of invasion or metastases.

There are 9 cases in which paragangliomas have occurred concurrently with neurofibromatosis. This concomitance may depend upon different degrees and directions of differentiation of neural crest cells.

Of the 5 original cases of paragangliomas which are described, one tumor was retroperitoneal and contained a brown pigment which was identified as melanin. The close chemical relationship between melanin and epinephrine is probably significant in this connection.

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DESCRIPTION OF PLATES

PLATE 192

FIGS. 1 and 2. Case 1. External and cut surfaces of paraganglioma.

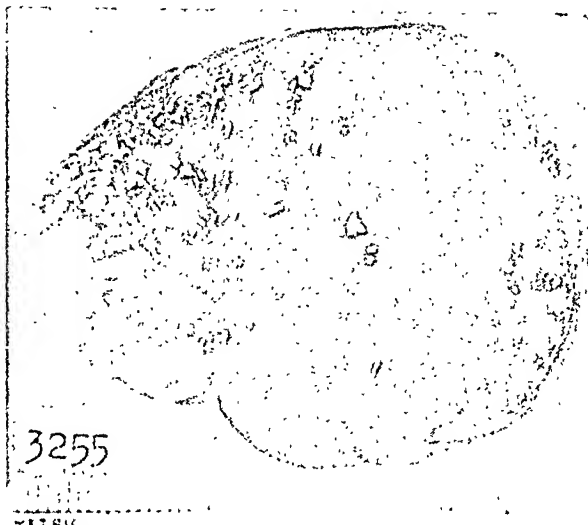
FIG. 3. Case 1. Pigmented area in tumor. Hematoxylin and eosin stain. $\times 240$.

FIG. 4. Case 1. Photomicrograph of nonpigmented area in tumor. Hematoxylin and eosin stain. $\times 240$.

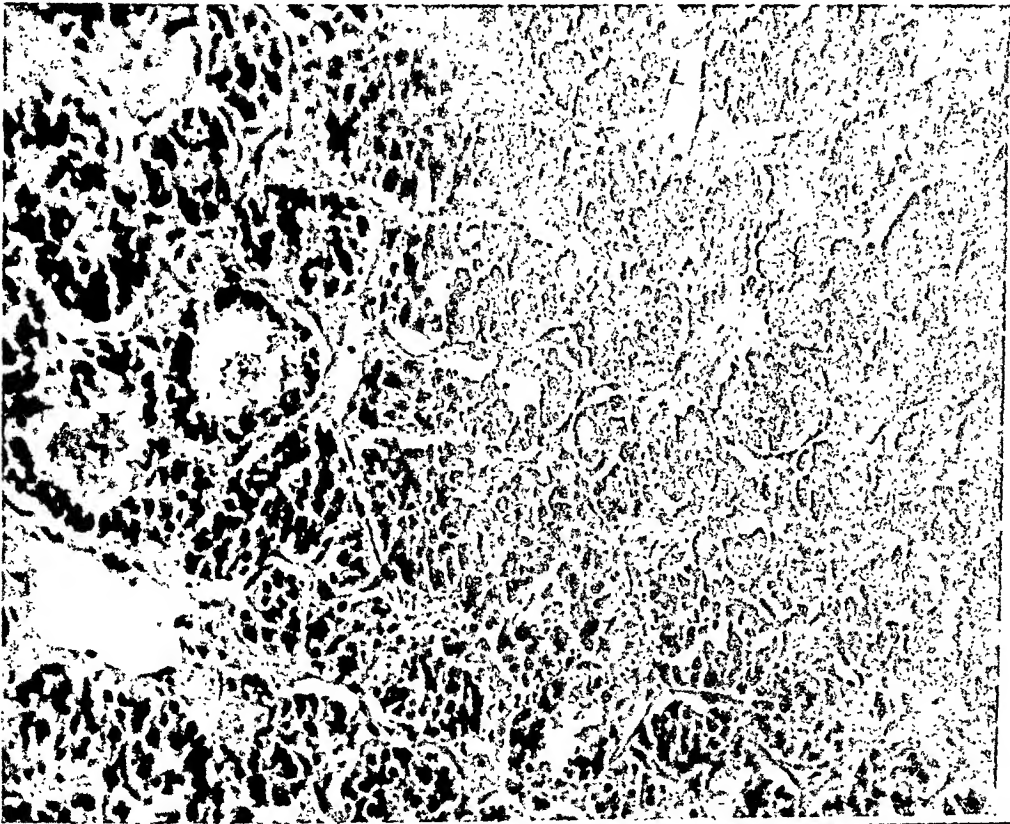
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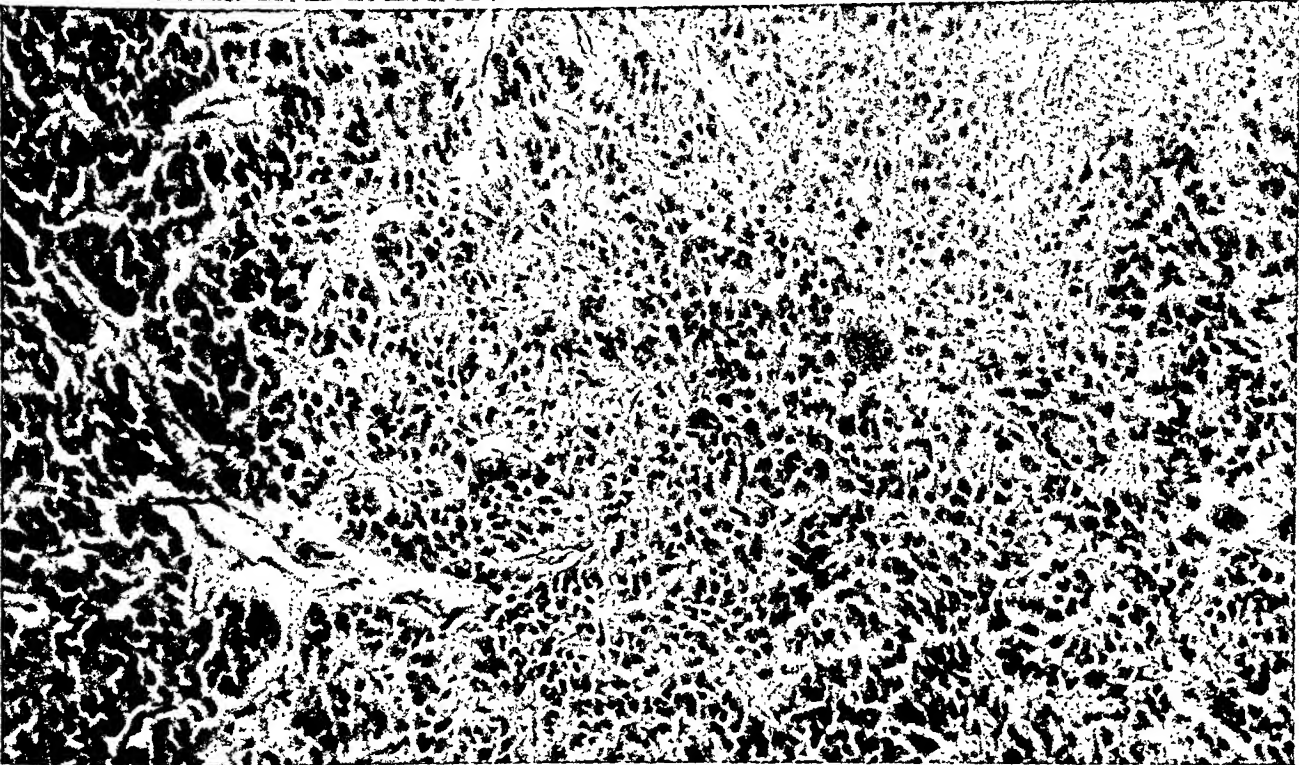


PLATE 193

FIG. 5. Case 2. Paraganglioma of right adrenal; diffuse hypertrophy of opposite adrenal.

FIG. 6. Case 2. Photomicrograph of representative area of the neoplasm. Hematoxylin and eosin stain. $\times 240$.

FIG. 7. Case 2. Cytoplasmic granules and prominent nucleoli in tumor cells. Azocarmine stain. $\times 516$.

5



6

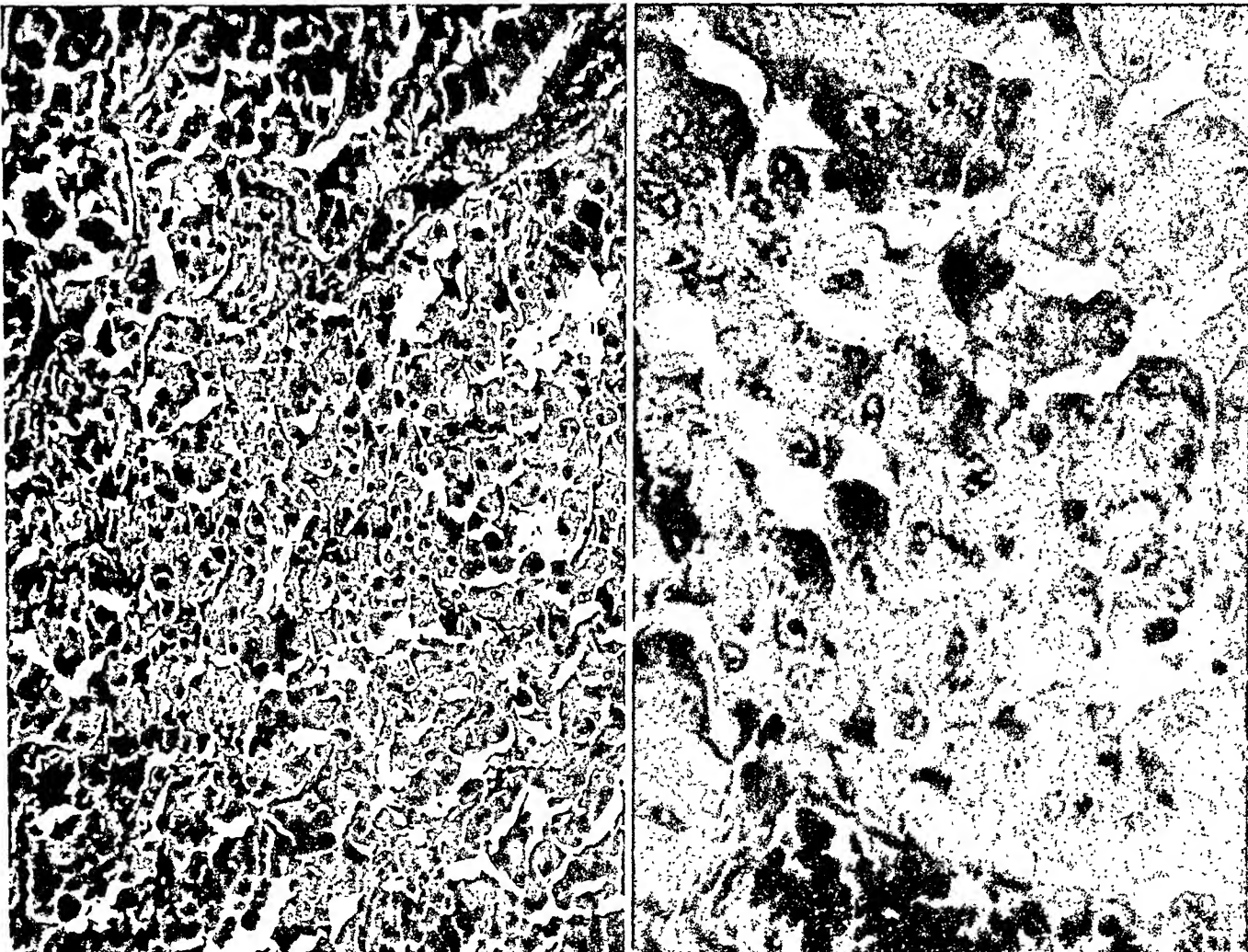


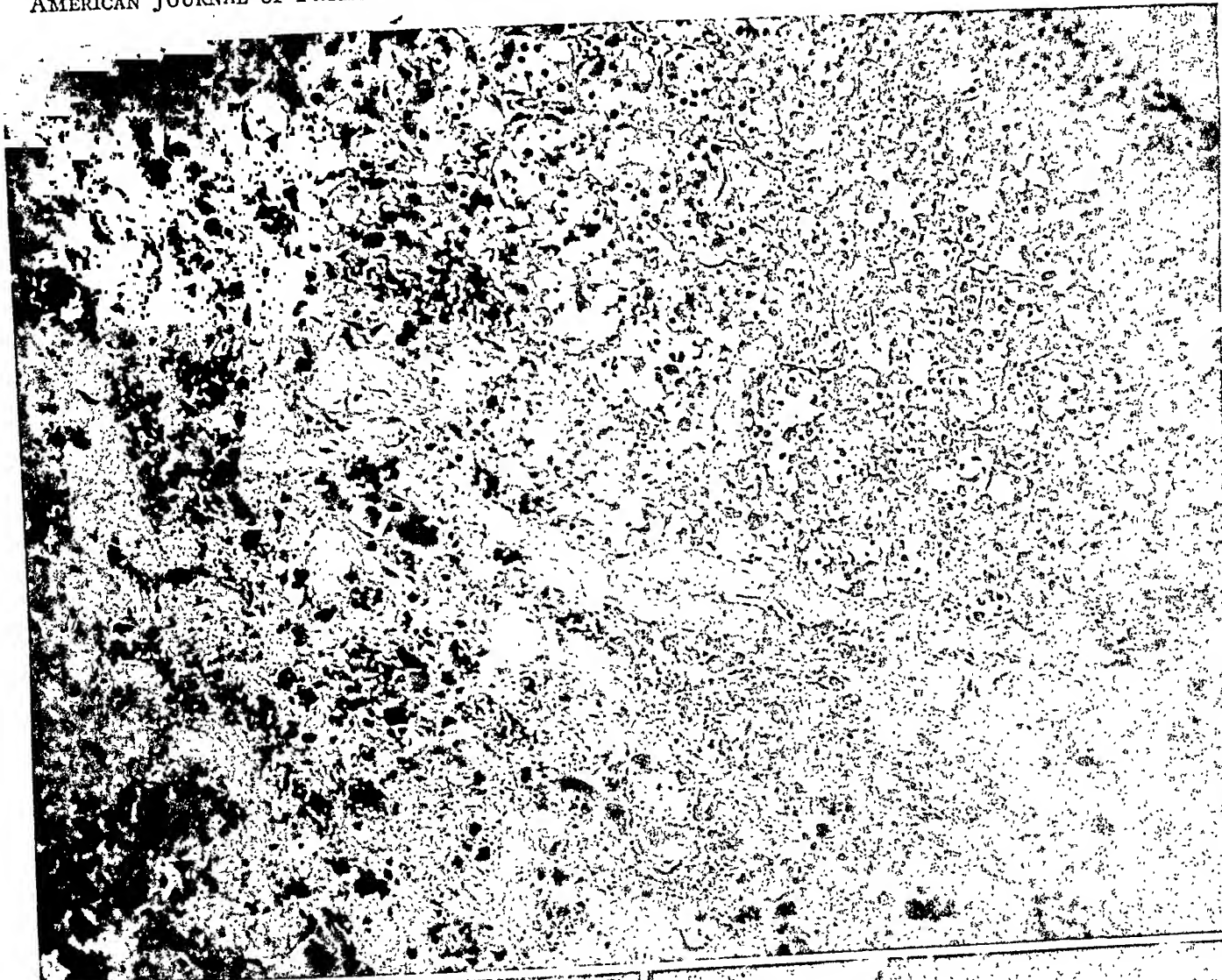
PLATE 194

FIG. 8. Case 3. Photomicrograph of neoplasm showing hydropic cell area at upper right and granular cell area at left. Hematoxylin and eosin stain. $\times 120$.

FIG. 9. Case 3. Multilocular vacuolation of cytoplasm in hydropic area. Hematoxylin and eosin stain. $\times 516$.

FIG. 10. Case 3. Granular cytoplasm in darker staining areas. Hematoxylin and eosin stain. $\times 516$.

8



9

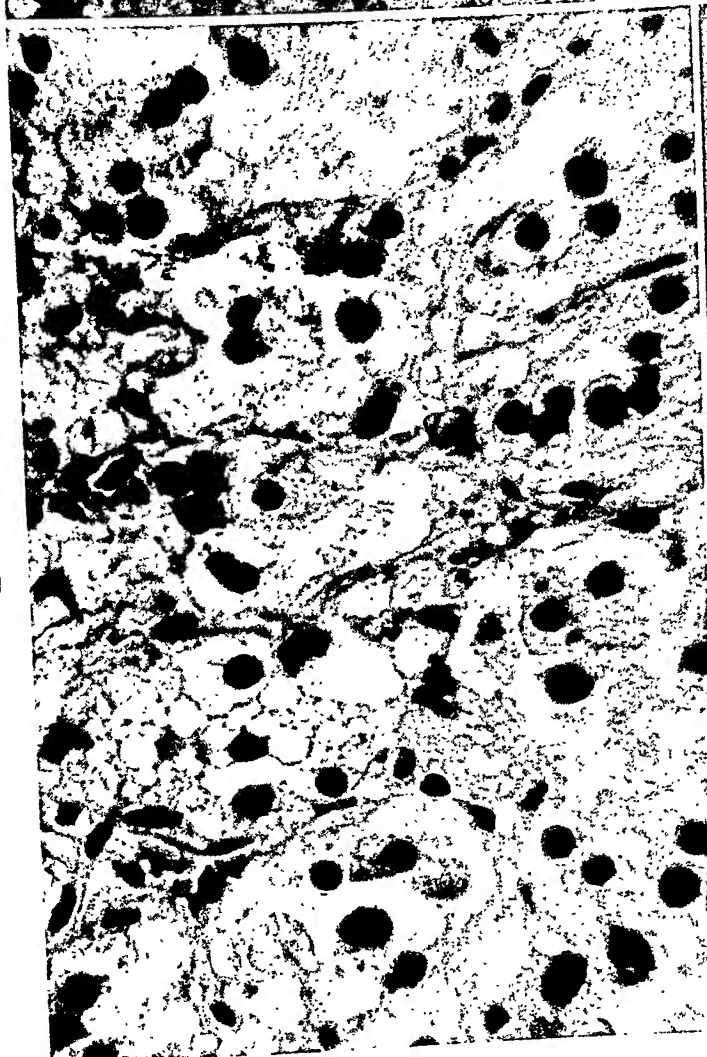


PLATE 195

FIGS. 11 and 12. Case 4. Photomicrographs of representative areas. Many oval and spindle-shaped cells; some pleomorphism and prominent vascular spaces. Hematoxylin and eosin stain. $\times 125$ and $\times 512$.

FIG. 13. Case 5. Photograph of paraganglioma of left adrenal gland.

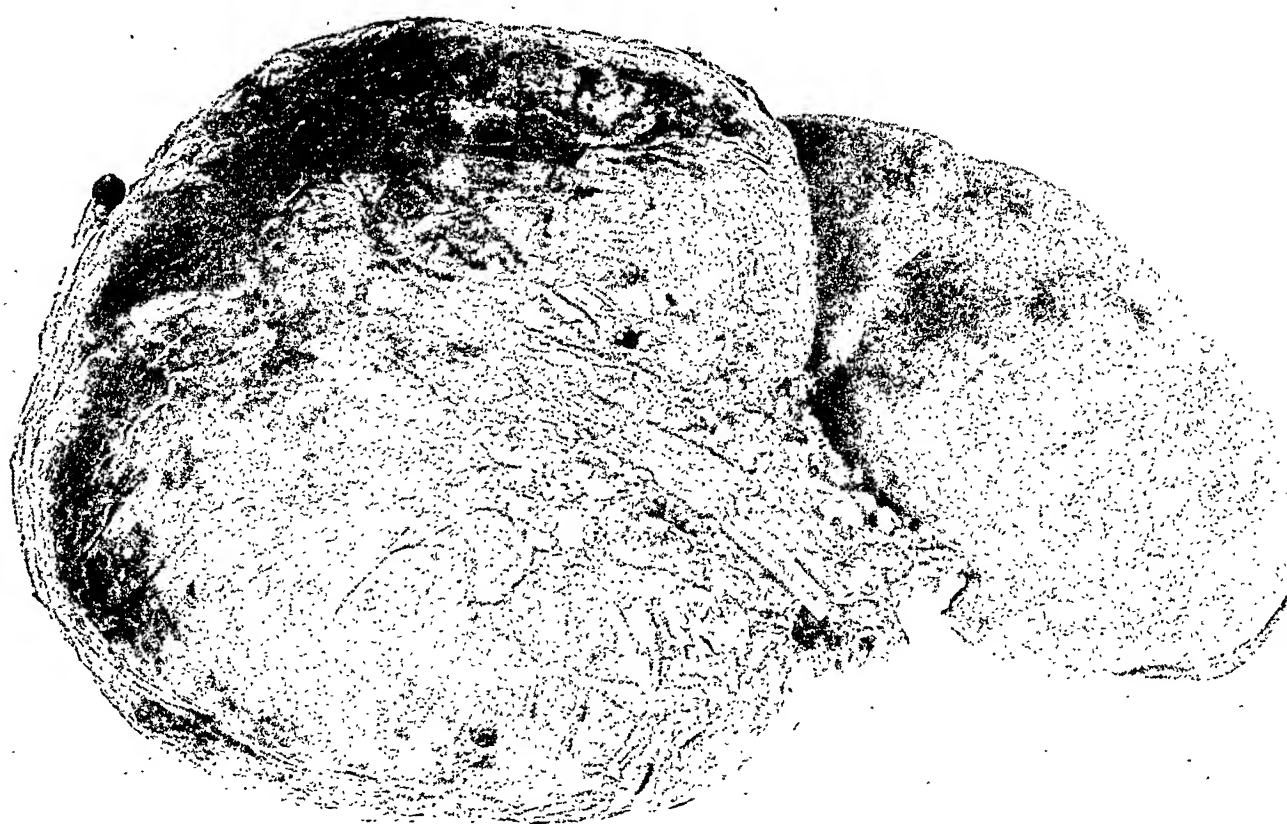
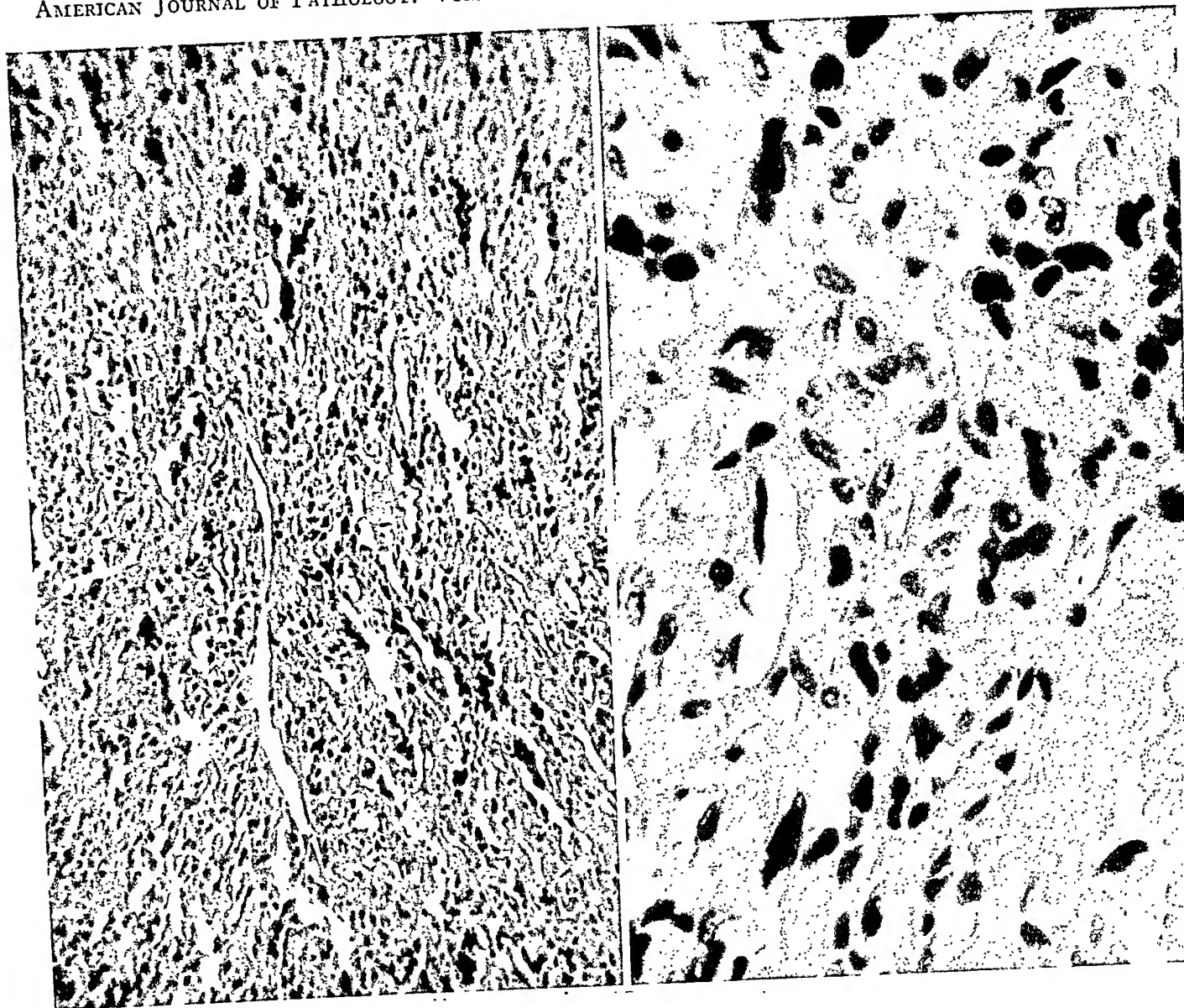
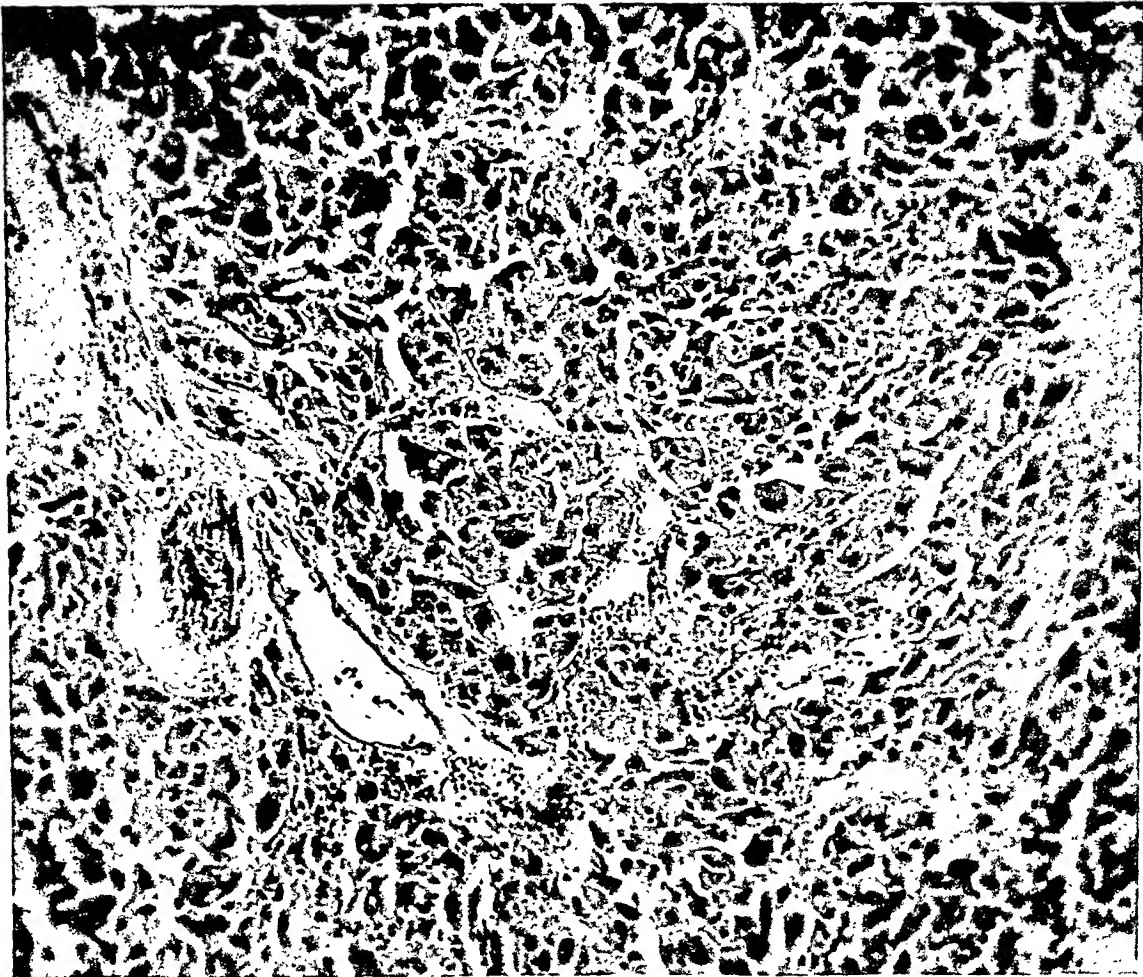


PLATE 196

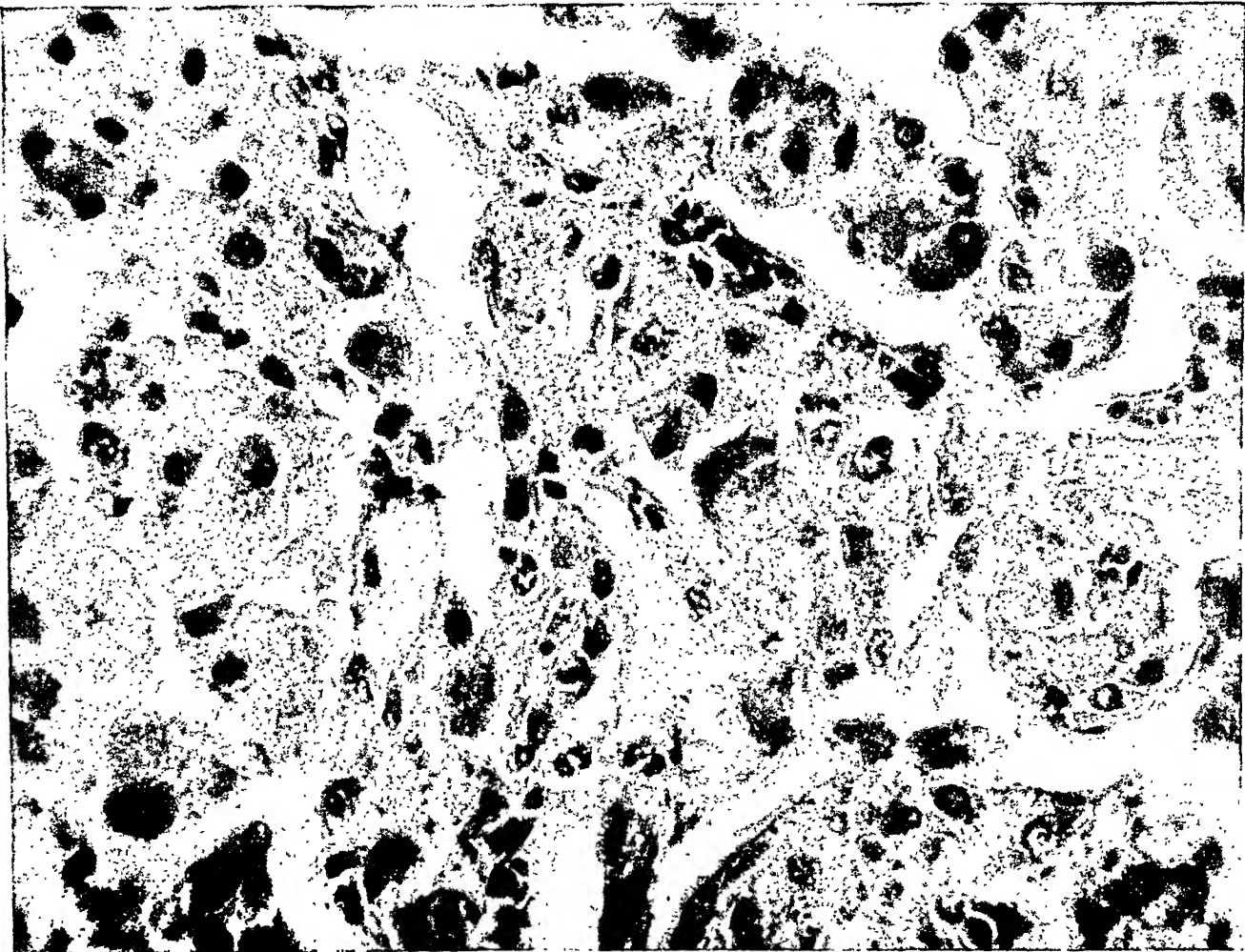
FIG. 14. Case 5. Photomicrograph of representative area of the neoplasm. Hematoxylin and eosin stain. $\times 240$.

FIG. 15. Case 5. Distinct granules in cytoplasm and wide sinus-like vascular spaces. Hematoxylin and eosin stain. $\times 512$.

14



15



THE CYTOLOGIC FEATURES OF CARCINOMAS AS STUDIED BY DIRECT SMEARS *

ERIK HAUPTMANN, M.D.†

(From the Department of Pathology, Duke University School of Medicine,
Durham, N.C.)

During the past 20 years the method of diagnosing cancer by visual examination of a single cell or a group of isolated cells has received more and more attention.¹⁻⁵ The method is based upon the assumption that morphologic as well as functional differences distinguish cancerous from noncancerous cells. The efficacy of the cytologic approach to the problem of tumor diagnosis is not yet proved. Its limitations and advantages are in part unknown, and only by controlled examination of large groups of tumors and normal tissues and experimentation with different methods will they be established. Therefore, this study was undertaken.

Almost since the advent of Virchow's cellular pathology attempts have been made to identify the characteristics of the cancer cell. The older work in this field was comprehensively reviewed by Quensel^{4,6,7} (1928) with particular emphasis upon the cytologic features of malignant cells in exudates. In the recrudescence of interest in this method of diagnosis, MacCarty^{2,8-10} with his group¹¹⁻¹⁴ in the United States and Dudgeon^{1,15,16} with others¹⁷⁻¹⁹ in England have been leaders in their respective countries. The development of the technic of needle biopsy of tumors²⁰⁻²⁶ and the work of hematologists in the study of bone marrow,²⁷⁻³¹ lymph nodes,³²⁻³⁹ and spleen⁴⁰ have all lent further impetus in this direction. Particular recognition must be extended to Papanicolaou^{3,41,42} and others,⁴³⁻⁵⁴ who in recent years have cultivated the method of single cell diagnosis to a point where it has found widespread practical application, particularly in examination of body excretions.

Despite this mass of evidence, the value of cytologic diagnosis of tumors is not widely acknowledged. The greatest skepticism is found among pathologists, and not without reason. Although there is a great difference between Borst's⁵⁵ statement, "Die Geschwulstzelle, auch die bösartige, hat weder in morphologischer, noch in chemischer, noch in irgend einer anderen Hinsicht etwas absolut Charakteristisches an sich," and that of MacCallum,⁵⁶ "No doubt, in time we shall have a reliable morphological criterion by which we may say definitely that an isolated cell is a cancer-cell or a normal cell," both indicate that the problem of single cell diagnosis of cancer is still to be settled. Rea-

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† Rockefeller Foundation Fellow, from the University of Zagreb, Zagreb, Yugoslavia.

sons for this skepticism are found in the small number of tumors dealt with in most of the reports, the great variety of tumors within the small groups, the diversity of methods used, and often the inadequate number of photomicrographs, limiting comparison of results.

Detailed descriptions of cytologic characteristics, such as have been reported in bone marrow studies³¹ and more recently in investigations of lymph nodes^{36,39} and spleens,⁴⁰ have not been recorded for malignant tumors. As a consequence, cytologic descriptions of tumors are limited to generalities such as anisocytosis, peculiar structure of chromatin and hyperchromasia of nucleus, size of nucleoli, or emphasis upon a detail (Quensel,^{4,6,7} MacCarty^{2,8-10}) which may have statistical significance but is of little help in the interpretation of a given case.

In contrast is the work of Papanicolaou³ and his followers.^{45,46,48-50,52,54} Utilizing a standardized technic and concentrating upon the cells of body excretions (vaginal, bronchial, etc.), they have demonstrated the usefulness of cytologic diagnosis of carcinomas.

From the above it appears that the most fruitful approach to this problem would be the examination of a large number of tumors, using a standardized technic and cataloging the changes which would make it possible to differentiate malignant from nonmalignant cells, regardless of origin. Such careful studies might eventually reveal differences permitting further classification, *i.e.*, carcinoma, sarcoma, or perhaps within a certain group even a detailed diagnosis, as is possible today with histologic methods (adenocarcinoma, squamous cell carcinoma, etc.).

It is in this manner that the present study was carried out. The tumors examined were limited to histologically proved carcinomas from various sites, and search for answers to the following questions guided the investigation:

1. What are the cytologic characteristics of carcinomas studied by means of the smear technic? Or, rephrased, is it possible to distinguish between histologically recognized carcinoma and noncancerous tissue, using the cytologic method?
2. Is there a cell or cells which characterize carcinomas, and are such cells always absent in noncancerous conditions?
3. Is there any relationship between the organ from which the tumor arises and the type of carcinoma cells? That is, do the cells of a carcinoma of the breast differ from those of a carcinoma of the stomach or skin?
4. Is there any relationship between the structure of a given carcinoma (adenocarcinoma, squamous cell carcinoma) and its cytologic features? In other words, are the cells which make possible the cyto-

logic diagnosis of carcinoma the same in an adenocarcinoma as in a squamous cell carcinoma?

5. Is the cytologic picture (smear) of a metastatic tumor the same as that of the primary tumor?

TECHNIC

The ideal method is to prepare smears of cells immediately upon removal of the tissue by the surgeon. Unfortunately, this was not possible in the majority of cases. Thus, the first problem was to determine what cellular changes occur following removal of tissue from the body. Since Rohr and Hafter³⁰ have shown that the changes in bone marrow after death are progressive, all of the tissues used in this study were refrigerated (4.0° to 5.0° C.) as soon as possible in order to retard these autolytic changes. To determine what changes the cells undergo at this temperature and what medium might best preserve them, fresh specimens were placed in four test tubes: (1) Fresh tissue in formalin, (2) fresh tissue in 0.85 per cent solution of sodium chloride, (3) fresh tissue in human plasma, and (4) fresh tissue untreated. All of the tubes were refrigerated (4.0° to 5.0° C.) and their contents examined in smears at intervals of 1 to 2, 6, 12, 24, 48, and 72 hours. It was observed that cells placed in formalin showed great changes in their staining reactions in less than 1 hour; those areas to which formalin had not penetrated (center of specimen) showed no tinctorial change. The cells in a physiologic solution of sodium chloride revealed remarkable changes within the first 12 hours—a fact noted many years ago by Forkner.³² The nuclei became swollen, the chromatin structure assumed an irregular, wide-meshed, net-like appearance, and the nucleoli became more visible. Finally the cell disintegrated. Cells suspended in human plasma, or untreated, showed almost no changes in staining reaction, cell form, or shape up to 24 hours at refrigerator temperatures. After this, they usually changed slowly, but in a manner different from the cells suspended in physiologic solution. Because of this experience all tissues which could not be examined immediately were placed under refrigeration in human plasma or untreated.

The second problem was to discover which method would best preserve the cell form and size, and produce an even distribution of cells in the preparation. The usual methods for making smears described in the literature^{1,31,36} and also touch preparations proved unsatisfactory for two reasons: (1) The cells were unevenly distributed and often were elongated in the direction of smearing; (2) tissues rich in mucus or fat (*e.g.*, mucosa of the gastro-intestinal tract, breast tissue) gave smears very poor in cells; the same was true of tumors which

contained much fibrous tissue. These methods, however, gave good results in material obtained from lymph nodes, spleen, and squamous cell carcinoma. Very poor results were obtained with the crush method^{20,23,25} of forceful spreading of tissue between two slides; too many clumps of cells were present to permit uniform staining or to preserve the shape of the cells.

The following method was found to be most satisfactory with all organs and tumors. A fragment of tissue, not larger than 2 to 4 mm. in diameter, was placed on a slide and covered with 3 or 4 drops of pooled human plasma. Using two needles, the tissue was teased apart until the plasma was clouded by the suspended cells. One drop was transferred to a coverslip and then covered with a second coverslip, producing an even distribution of the cell suspension. The coverslips were then pulled apart and dried. This method is similar to that used by some hematologists⁵⁷ in the preparation of blood smears.

The third problem was the selection of a stain. It was desired to preserve the cells as well as possible and, at the same time, to differentiate clearly the various cellular structures. Most of the methods described in the literature, judging from illustrations and descriptions, did not seem to give these desired results. Usually the chromatin of the nucleus was clumped so as to prevent recognition of the more delicate structures (Martin and Ellis'²² method with hematoxylin and eosin, Dudgeon and Barrett's¹ method with Schaudinn's solution and subsequent treatment with hematoxylin, iodine, and eosin). It was found impractical to use the more sensitive method of supravital staining described by Sabin⁵⁸ or Quensel's⁴ method for examination of tumor cells, since in both cases it is necessary to use very fresh material, which was not always possible in this work. Two methods appeared to be better adapted to my purposes: The various combinations of Romanowsky stains currently in use in hematology,^{31,57} which show clearly the details of cells; and Papanicolaou's³ method, which has proved so popular in recent years in the examination of tumor cells of different body excretions (vaginal fluid, sputum, urine, gastric juice). Therefore, in this study several preparations of each specimen were stained, some with a combination of May-Grünwald-Giemsa's stain, others with Wilson's* stain, and still others by Papanicolaou's method (hematoxylin-EA₂₅-OG₆). Of the two hematologic methods, eventually only Wilson's stain, because of its simplicity, was retained, although both methods gave equally good results. The material stained by Papanicolaou's method was prepared upon slides, according to his directions.³ In comparing the two methods (of Wilson and of Papanic-

* Phosphate buffered Wright's stain.

olaou), the details, particularly of the nucleus (chromatin structure), are more clearly differentiated by Wilson's stain, although Papanicolaou's method was sometimes more valuable for studying cell outlines. Specimens containing much fat, mucus, or colloid were likewise better stained by Papanicolaou's technic. Wilson's stain, on the other hand, had the advantage of bringing into greater contrast the nucleus (violet) and nucleolus (blue), as well as nucleus (violet) and cytoplasm (light blue to dark gray-blue). With Papanicolaou's method most of the tumor cell cytoplasm was stained green, the nucleus grayish violet. The nucleolus, although readily visible, appeared only as another variation of the same gray-violet; the chromatin was more distorted than when Wilson's stain was used. Both methods have been used to complement each other in almost every case.

MATERIAL

In accordance with the questions stated in the introduction, two classes of material were examined: Histologically proved carcinomas, and normal and noncancerous (inflammatory, benign tumor, etc.) tissues from various sites.

A total of 188 cases have been examined; 78 in the first group (carcinoma), and 110 in the second (control) group, noncancerous. From the 188 cases, 268 organs or tissues have been examined; 178 were noncancerous, 90 fell into the carcinoma group. They have been individually classified. For instance, if in a case of carcinoma of the cardia of the stomach, the tumor, the grossly unchanged mucosa of the fundus, and a mesenteric lymph node were examined, each area was separately classified as noncancerous or carcinomatous according to its histologic findings. In the same way two or more organs or tissues from a case without a carcinoma were individually examined and classified.

The noncancerous tissues are listed in Table I, and the carcinomas, according to the organ from which they were taken, in Table II. It must be emphasized that the classification in Tables I and II is based not upon cytologic, but upon histologic, examination. In this manner each organ or tissue was appropriately cataloged as either noncancerous or carcinomatous.

RESULTS

It is not intended to describe in this paper the cytologic features of noncancerous organs or tissues in smear preparations, except in so far as it may be necessary to elucidate the differences between noncancerous processes and carcinoma. On the other hand, it is necessary to describe the cytologic characteristics of those tissues which have been classified histologically as carcinoma.

The over-all cytologic picture of the carcinoma smears shows certain characteristics which occur repeatedly. These characteristics, which may be subdivided, suggest a possible cytologic classification. Five types could be distinguished among the smears of 90 carcinomas: The squamous cell type, the columnar cell type, the round cell type, the undifferentiated cell type, and the oat cell type.

Carcinoma Smears of Squamous Cell Type (Fig. 1)

In carcinoma smears of squamous cell type, squamous cells were present in varying numbers, just as in smears of normal skin or mucous membranes (Fig. 6); most of the cells, however, were atypical, varying in size, shape, and color. Instead of the usual polygonal form with

TABLE I
Tissues Histologically Classified as Noncancerous

Organ (tissue)	Number of specimens examined
Esophagus	2
Stomach (corpus and fundus, 8; pylorus, 2)	10
Duodenum	1
Jejunum	2
Ileum (no lesion, 1; inflammation, 2)	3
Colon	9
Rectum	5
Diverticulosis of intestine	1
Polyp of colon	1
Bronchus	1
Lung (no lesion, 1; lung abscess, 3)	4
Uterus (endometrium, 22; cervix (cervicitis), 26)	48
Ovary	9
✓ Breast (chronic mastitis, 1; granulomatous lesion, 1; cystic hyperplasia, 2; fibrosing adenomatosis, 2; comedo-adenoma, 1; intracanalicular fibro-adenoma, 3; adenofibroma, 1)	11
Testis	2
Benign prostatic hypertrophy	4
Kidney (pyelonephrosis)	1
Bladder	1
Submandibular gland	1
Mixed tumor of parotid	1
Gallbladder (no lesion, 1; cholecystitis, 5)	6
✓ Thyroid (cystic nodular goiter, 1; nontoxic adenoma, 1; lymphadenoid goiter, 1; hyperplasia and hypertrophy, 2; fetal fibro-adenoma, 1; nodular colloid goiter, 1; embryonal adenoma, 1; nodular goiter with fetal adenoma 1; colloid goiter, 2; nodular thyroid, 1; nodular goiter with hyperplasia and colloid nodules, 1)	13
✓ Lymph node (no lesion, chronic inflammation, Boeck's sarcoid)	16
Spleen	7
Skin	2
Striated muscle	1
Fibromyoma uteri	3
Sinusitis maxillaris	1
Nonspecific inflammation	6
Epiglottis	1
Lipoma	2
Papilloma of abdominal wall	1
Vagina	1
Giant cell epulis	1
Total	178

a dense, dark, almost pyknotic nucleus and abundant cytoplasm which stains light pink or bluish, the cells often were elongated and stained darker blue, but were still pale; the nucleus sometimes was larger than is normal, and the chromatin might be net-like. These cells were readily recognized as variations from the normal squamous cell. Figures 7 and 8 depict a group of cells which showed a close relationship to the

TABLE II
Neoplasms Histologically Classified as Carcinoma Listed by Location

Location	Number of specimens examined
Lip	3
Mouth	2
Esophagus	1
Stomach	9
Colon	7
Rectum	8
Larynx	1
Lung	4
Mediastinum	1
Uterus (endometrium, 3; cervix uteri, 6; infiltrating from ovary, 1)	10
Ovary (primary, 4; metastatic, 1) ¹ / ₂	5
Vulva	2
✓ Breast	12 —
Pancreas	1
✓ Thyroid	1 —
Kidney	1
Bladder	1
Peritoneum (metastatic)	2
Neck	1
✓ Lymph node (metastatic)	13 —
Abdominal wall (metastatic)	1
Chest wall	1
Skin	3
Total	90

squamous cells described above. Their cytoplasm was bluish, sometimes blue, almost always well outlined; rarely, one or two borders merged gradually with the background. The size of the cell and its shape were more or less the same as those of the previously mentioned cells; sometimes they were slightly larger. Elongated forms and spindle-like types were more common than in the former. The nucleus, however, was always larger, varying from the size of a large lymphocyte (8 to 10 μ) to 15 μ or more in diameter. When the nucleus was large, it formed the greater portion of the cell, so that the cytoplasm was relatively sparse. The nucleus, as a rule, was well delineated and round, rarely slightly oval. The chromatin formed a regular, medium-sized meshwork. It contained one nucleolus, rarely two or three, each 2 or 3 μ in diameter. Nucleoli were usually round and blue or bluish; exceptionally they were dark blue and slightly irregular. This cell is designated a "nucleolated squamous cell." In some instances cells were

seen which resembled those found in the deeper layers of normal epithelium (basal cells, according to Papanicolaou³). They usually appeared in groups; single cells were seen rarely. These cells are henceforth termed "malignant epithelial cells" (Figs. 9 and 10). They varied in size (10 to 20 μ), and were rhomboid or polygonal. The cytoplasm was dark blue and rather sparse; it was not very sharply outlined, but the border was still visible. Sometimes keratin formation, as described by some authors,²⁰ was seen in the cytoplasm in the form of red granules, small rods, or dust. The main mass of the cell usually was constituted by the nucleus, which was oval to round and usually slightly irregular; large forms predominated. The chromatin was granular, irregular, rather coarse, amorphous, and often was condensed in the form of a ring at the periphery of the nucleus. The nucleoli were multiple (one to four), dark blue, irregular, poorly outlined, and measured 1 to 3 μ or more in diameter. Another cell seen in smears of this type was a giant cell (Fig. 13), which always appeared singly. Exceptionally as many as three or four per low-power field might be seen. The sparse cytoplasm, which stained dark blue, usually was present only at the poles of the nucleus, disappearing gradually without sharp borders at the periphery. Sometimes the cell contained small cytoplasmic vacuoles. The nucleus was large (20 to 40 μ) and usually irregular because of many indentations. It possessed numerous nucleoli, which were blue; but, because of the presence of small granules of dense chromatin which produced a turbid appearance, the nucleoli often were difficult to see. A fibroblast-like cell (Figs. 11 and 12) was frequently seen in these smears. It usually was larger than true fibroblasts. The cytoplasm was darker, often dark gray-blue, poorly outlined and usually fringed at its margin. The nucleus was long, oval, and narrow; the chromatin was net-like, its strands sometimes thick and irregular; the nucleoli were light blue to blue. The term "pseudo-fibroblast" will be used to identify this cell. These cells varied in size and sometimes showed transitional forms to the above-mentioned giant cells and nucleolated squamous cells; on the other hand, it frequently was difficult to distinguish them from fibroblasts. Such cells have been described by Papanicolaou³ and others in vaginal smears of carcinoma of the cervix. Comparing the smears with the histologic preparations led to the opinion that they probably are not fibroblasts. It is more probable that these spindle cells are derivatives of epithelium. In connection with this it should be mentioned that in experimental tumor transplantation occasionally a carcinoma will show sarcoma-like changes, that is, the appearance of spindle cells. The explanation (Ewing⁵⁹) is that the spindle cells represent derivatives of epithelium, rather than a change in the character of the tumor (carcinoma to sar-

coma). Besides cells of the four types mentioned above, in carcinoma smears of squamous cell type were found erythrocytes, leukocytes, fibrocytes, and fibroblasts. (For their description see Tischendorf.³⁹)

One or another cell form, as described above, may predominate in the smear. Basically, however, the smears show a very similar appearance. Smears of this type were found in cases of carcinoma of the lip, mouth, esophagus, larynx, bronchus, cervix uteri, vulva, kidney, skin, and in some metastatic carcinomas in lymph nodes. In one case of cervical "carcinoma *in situ*," only such cells as are illustrated in Figure 10 (malignant epithelial cells) were seen.

Carcinoma Smears of Columnar Cell Type (Fig. 2)

The columnar cell (Figs. 14 and 15) in all its variations characterized this type. In its usual form it measured approximately 6 by 15 μ , possessed a round to oval nucleus, and light blue to blue cytoplasm with well demarcated outlines. Sometimes red, dust-like granules were visible at one end of the cytoplasm. Among these cells were others which had larger nuclei. These sometimes were irregular; their chromatin was arranged in strands of varying thickness. Often small, irregular nucleoli were seen. The cytoplasm of these cells was poorly defined and dark grayish blue. Another cell similar in many respects to those just described was found only in carcinomas (Fig. 16). It, too, was more or less columnar, showed the same variations as the above described cells, with the addition that usually two or three irregular, dark blue nucleoli were present. Where only one nucleolus was found, it was large and round. The nucleoli might occasionally be pale but retained all the other properties described. This cell was named the "malignant columnar cell." The third cell type, seen only in carcinomas, was the "undifferentiated cell" * (Fig. 19) with no cytoplasm or with a little cytoplasm in the form of a narrow, perinuclear, light grayish zone. The nucleus measured 12 to 20 μ in diameter. It usually was round and irregular with net-like chromatin, the strands of which were rather thick, sometimes forming wide meshes. At other times the chromatin was more granular in appearance. The nucleoli were large, pale blue, irregular, and often multiple. Large round cells were seen also in smears of this type (Fig. 17). They usually were round and relatively well delineated. The nucleus usually was about 12 to 18 μ in diameter, round, and predominantly regular. The chromatin did not form strands in most of these cells, but usually was amorphous in appearance and irregularly distributed, producing darker and lighter areas. The nucleoli were round, dark blue, and often masked by the dense chromatin, so that their borders were not clearly visible.

*The term "undifferentiated cell" is one of convenience. Whether it is cytogenetically less differentiated than the other cells is not known.

In other cases in which the chromatin formed an irregular, coarse net, the nucleoli were seen readily and were round, large (3 to 8 μ), and blue. The cytoplasm was bluish or blue and well preserved in many of these cells; in some, however, its margins faded gradually into the background. These cells, examined under low power, frequently resembled plasma cells, at first glance. The large, round cells might achieve considerable size so that they formed giant cells (Fig. 18) comparable in magnitude to those seen in the squamous cell smear. However, the cytoplasm of these cells, in contrast to that of the giant cells of the squamous cell smear, surrounded the nucleus and was not localized largely at the poles. This appearance suggested that the cells were derived from the large round cells. Giant cells, as illustrated in Figure 13 and described in connection with smears of the squamous cell type, were seen also in the smears of the columnar type. The same was true of the pseudofibroblast (Fig. 12). Both of these forms were rare in smears of this type, as compared to those of the squamous cell type. In addition to the above-mentioned cells, leukocytes, erythrocytes, fibroblasts, fibrocytes, and macrophages were present.

Carcinoma smears of the columnar cell type have been found in carcinomas of the stomach, colon, rectum, and ovary, and in some metastatic carcinomas of lymph nodes.

Carcinoma Smears of Round Cell Type (Fig. 3)

In some cases classified as carcinoma of round cell type, large, round cells (Figs. 17 and 18) composed the entire smear; in other cases some columnar cells (Fig. 16) were present. The impression, in the latter event, might be that of a smear of the columnar type with predominance of large, round cells. In other instances no columnar cells could be seen. In addition, a few undifferentiated cells might be visible.

Smears of this type have been seen chiefly from carcinomas of the cardia of the stomach and in some carcinomas of the breast, lung, pancreas, bladder, and uterus. This type was seen twice in smears of metastatic carcinoma.

Carcinoma Smears of Undifferentiated Cell Type (Fig. 4)

What has been said of smears of the round cell type may also be repeated in a description of the undifferentiated cell type, with the difference that the predominating cell was that previously described as the "undifferentiated cell" (Fig. 19). Smears which revealed such a picture have been obtained from carcinomas of the stomach, colon, rectum, ovary, and breast.

Carcinoma Smears of Oat Cell Type (Fig. 5)

The word "oat" is used for this type because the characterizing cells resembled those found in the sputum of patients with carcinoma of the respiratory tract and described as oat cells. Two forms of small cells were seen in this type. One represented cells which could not be differentiated from those of normal organs or tissues. The nucleus was the size of that of a small to large lymphocyte with little variation in size and shape; it was round to oval. The chromatin usually was dense, sometimes granular and irregularly distributed. Nucleoli were very rare. No cytoplasm was present, or, if present, it appeared only as a narrow bluish zone about the nucleus. The cells usually appeared in large sheets and might be compared to the type which Rohr and Heggin²⁹ have designated "kleinzelliger Carcinom Typ" in bone marrow. There appears to be justification for their opinion that a diagnosis may not be made on the basis of a single cell, but only from cells which occur in the form of sheets. Whether the latter is true in every case is, in my opinion, open to question, because in some smears of chronically inflamed spleens and lymph nodes similar cells have been found, often in small groups.

The second small cell is that described in the literature¹⁶ as the oat cell of bronchial carcinoma. It was similar to the first small cell, except that it possessed a definite but narrow rim of blue cytoplasm. The cytoplasm formed a short bipolar process. This cell likewise appeared frequently in sheets, but might sometimes be recognized when isolated. Finally it should be mentioned that, just as in the smears of the first two types, other cells were present: erythrocytes, leukocytes, fibroblasts, fibrocytes, and macrophages.

Smears of this type were seen in one case of carcinoma of the thyroid, in a case of pulmonary carcinoma, and in almost half of the proved metastatic carcinomas in lymph nodes.

Table III presents the different types of smears and the histologic classification of the carcinomas from which the smears were made.

DISCUSSION

1. *What are the cytologic characteristics of carcinomas studied by means of the smear technic? Is it possible to distinguish between histologically recognized carcinoma and noncancerous tissue using the cytologic method of study?*

The most widespread and accepted method of cancer diagnosis—histologic study of tissue—is based upon the recognition of several changes in structure, many of which are cytologic: increase in the number of cells, marked variation in their size and shape differing from

the original cell type, increase of the chromatin mass, large nucleoli, multiple and abnormal mitotic figures, loss of polarity, and infiltration of the surrounding tissue (Aschoff,⁶⁰ Ewing,⁵⁹ MacCallum⁵⁶). All may be studied by cytologic methods except the last, which is often considered the most important. Since cytologic technics preserve the components of a cell in greater detail than the methods of histology, vari-

TABLE III
Types of Carcinoma Smears with Corresponding Histologic Classification

Type of smear	Histologic classification
Squamous cell type, 29	Squamous cell carcinoma (lip, 3; mouth, 2; esophagus, 1; larynx, 1; lung, 1; cervix uteri, 4; vulva, 2; lymph nodes, 5; skin, 2) Carcinoma (bronchogenic, 2; kidney, 1; neck, 1; cervix uteri (<i>in situ</i>), 1) Tissue with degenerating neoplastic(?) cells (skin, 1) Benign* (chronic cervicitis, 1; cervix with no lesion, 1)
Columnar cell type, 22	Adenocarcinoma (stomach, 4; colon, 5; rectum, 6; endometrium, 1; uterus, 1; ovary, 2; lymph node, 2) Malignant adenoma (uterus, 1)
Round cell type, 13	Adenocarcinoma (stomach, 3; cervix uteri, 1; endometrium, 1; lymph node, 1) Carcinoma (lung, 1; pancreas, 1; bladder, 1; mediastinum, 1; duct cell of breast, 1; canalicular of breast, 1; medullary of breast, 1)
Undifferentiated cell type, 14	Adenocarcinoma (stomach, 2; rectum, 1; colon, 1; peritoneum, 2; abdominal wall, 1; ovary, 3) Carcinoma (rectum, 1; chest wall, 1; scirrhus of breast, 1; medullary of breast, 1)
Oat cell type, 9	Adenocarcinoma (thyroid, 1) Carcinoma (colon, 1; lymph node, 5) Benign* (nodular hyperplasia and fibrosing adenomatosis of breast, 1; cystic hyperplasia of breast, 1)
No finding, 4	Carcinoma (canalicular of breast, 2; scirrhus of breast, 2)

*These cases will receive further comment.

ous cytologists from time to time have selected one or more of the above characteristics as their criteria for cancerous changes.

The opinion popular among older authors (literature by Quensel⁷) was that cancerous cells are larger than normal cells, and that the ratio of nucleus to cytoplasm is changed in favor of the former. This observation also has been made more recently,^{3,38} but the change is considered suggestive, rather than characteristic, of malignancy. My smears showed that the majority of carcinomas do have larger cells than those of the tissue from which they arose. It appeared impossible, however, to make a diagnosis of malignancy on the basis of the size of a cell or the ratio of nucleus to cytoplasm.

Quensel^{4,6,7} studied malignant cells in body fluids using the method of supravital staining. He stated that the nucleolus is of diagnostic importance. In his cases of carcinoma the nucleoli varied in diameter from 3 to 10 μ ; exceptionally they were smaller, 1.5 to 2 μ . In contrast, the nucleoli of endothelial cells (control) measured 1 to 1.5 μ , very occasionally 2 to 3 μ . He showed that the size of the nucleolus in carcinoma is independent of that of its nucleus. This is indicated by the ratio, nucleolus:nucleus, which is 0.20 to 0.60 in carcinoma, as compared to 0.14 to 0.20 or less in endothelial or other cells. Further characteristics of malignancy, which Quensel considered less important, are giant vacuoles (larger than 40 μ) and irregular sheet formation with partial or total overlapping of nuclei, as compared to other cells in body fluids in which the vacuoles are smaller and the cells form "plaques." The change in the size of nucleoli was not found in sarcomas. Quensel's results were confirmed by Zadek and Karp⁶¹ and Zadek,^{62,63} who studied tumors of pleura, peritoneum, lung, and urogenital tract. MacCarty and his co-workers,^{2,8-10,13} using a different method, arrived at similar results showing that the ratio of the nucleolar to nuclear surface, estimated as an average from a large number of cells (25 to 100), was always greater in malignant processes than in any other lesions of a particular organ (tissue). This ratio varied from 1:5 to 1:17 in malignancy and from 1:13 to 1:45 in control cases; they considered this ratio to be of diagnostic value. Rohr and Hegglin²⁹ showed that in the bone marrow only a certain number of carcinomas possess the cell characteristics which Quensel ascribed to them. Other carcinoma cells may have small nucleoli or even none. Thus, while it may be that a large nucleolus or a certain ratio of the nuclear diameter (surface) to that of the nucleolus may be characteristic of carcinoma cells in body fluids, it is not necessarily true for carcinoma cells in general. Similar observations have been made on my material. Quensel and MacCarty's observations seem applicable to a large number of my carcinomas (stomach, breast, and endometrium). Many others, however (lung, thyroid, and some of the breast carcinomas) did not fit into this scheme. The cells of metastatic tumors often had only small nucleoli or none.

Irregularity of cell shape and size, changes in the structure of chromatin, and atypical staining reactions of cytoplasm have been stressed by almost every author who has studied malignant tumors cytologically, either directly from the tumor or in excretions and body fluids. These changes have not been considered diagnostic, but only suggestive of malignancy. Similar changes can be seen in inflammatory lesions as well as in benign tumors, usually, however, to a lesser extent. The

same opinion may be expressed concerning irregular sheet formation with overlapping of nuclei. While this phenomenon is common in carcinoma, it can be seen also in other processes (inflammation). In summary, therefore, it may be said that many cellular changes suggest malignancy but are neither sufficiently constant nor sufficiently prominent to establish their diagnostic significance. When seen in a smear, they should, however, awaken the suspicion of the observer.

These suggestive changes as seen in my material are summarized in Table IV. As may be seen in this table, which is based on the study of smears from 90 carcinomas, there were certain cellular changes suggestive of a process which may not be benign. The size of the individual *cell* in carcinoma smears was, in general, larger than that in noncancerous processes; this was especially noticeable when the cells were compared with those of the tissue from which the carcinoma arose. Variation in size and shape might often be very marked, although some tumors showed a relatively uniform cell picture (instances of carcinoma of bronchus, carcinoma of thyroid). The cells were individually disposed or were present in sheets. When in the latter form, the nuclei were partially superimposed and polarity was lost to a greater or lesser degree, as compared with noncancerous processes, in which the "plaques" consisted of a single layer of cells, the nuclei of which were regularly arranged. There were, however, noncancerous processes which might show the same phenomena as carcinoma, but to a lesser extent. The adhesion of cells to form groups of various sizes and shapes was much more commonly seen in noncancerous epithelium than in carcinomas, in which most of the cells usually lay separated from each other.

The *cytoplasm* of the cells in carcinoma smears was either scanty or poorly outlined, merging gradually with the background. Few of the carcinomas possessed cells which were relatively rich in cytoplasm (nucleolated squamous cells, Fig. 7; some of the malignant columnar cells, Fig. 16). The cytoplasm varied from light grayish pink to blue and often dark gray-blue. Noncancerous cells usually were light blue to blue and, in addition, had more abundant and better outlined cytoplasm.

The same morphologic variations which were described in carcinoma cells, in general, can be said to characterize the *nucleus*. It was usually larger than that of the control group and sometimes attained giant dimensions (Figs. 13 and 18). It was round to slightly oval, often irregular, in contrast to the nucleus of noncancerous tissue, which was more regular. Anisonucleosis and poikilonucleosis were almost always present in the cytologic picture of carcinoma, rarely in the control

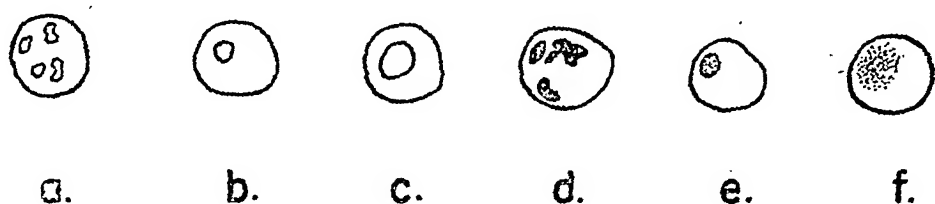
TABLE IV
Changes Suggestive of Malignancy Seen in the Smears of 90 Carcinomas as Compared with Those of 178 Control Tissues

	Findings based on examination of single cells		Findings based on examination of groups of cells	
	Noncancerous tissue	Carcinoma	Noncancerous tissue	Carcinoma
Cell	Regular in size and form; color light gray to blue	Irregular (anisocytosis and poikilocytosis); larger than the original tissue cell; color often dark gray-blue	Uniform appearance; in rows or "plaques"	Anisocytosis, poikilocytosis; in clumps and large sheets
Cytoplasm	Usually well outlined; moderate to abundant in amount	Poorly outlined; rarely well limited; often nuclei without cytoplasm; commonly little cytoplasm		
Nucleus	Round, oval, kidney-shaped; usually less than 15 μ in diameter	Round or oval, often irregular; usually 15 μ or more	Nuclei show regular arrangement	Nuclei cover each other and show loss of polarity; anisonucleosis and poikilonucleosis
Chromatin	Dense, clumped, or net-like, but regular	Irregular, coarse, net-like; irregular, granular; dusty; chromatin often forms a clearly visible ring at the border of the nucleus		
Nucleolus	None, or small to medium size, regular in shape; light blue or violet; rarely dark	Medium size to large or giant, often irregular; light blue to dark grayish blue		
Ratio of cytoplasm: nucleus	In favor of cytoplasm	Usually in favor of nucleus		
Ratio of nucleolus: nucleus		High		

group. When, therefore, a high degree of irregularity was present in a noncancerous process and a low degree in a carcinoma, they were indistinguishable on this basis.

The *chromatin* in the control group was dense and clumped; if net-like, it was regular and delicate. In carcinomas the chromatin was irregular, was composed of coarse strands, and formed a net-like structure. This was sometimes small-meshed, and at other times showed large, irregular meshes; both forms might be present in the same smear. In still other cases the chromatin formed a grid of coarse strands or consisted of irregular, coarse granules; it might have a dense, irregular, amorphous appearance. At times the chromatin was condensed in the form of a ring at the periphery of the nucleus.

The *nucleolus*, considered by many authors to be one of the most suggestive or even diagnostic indices of malignancy, particularly in carcinoma, was not visible in the majority of the cells of the control group. When present, it usually was not larger than 1 to 2 μ in diameter. It was regular and stained light blue or the same color as the chromatin. Exceptions, however, were found: in some of my specimens, identified histologically as progestational endometrium, the nucleoli were large and sometimes stained dark blue; the same was noted in some examples of atrophic endometrium, in tubular epithelium of a case of chronic pyelonephritis, and in the mucosal cells of a bladder with carcinoma. The smear from the last mentioned was obtained from an area reported histologically as showing "no lesion." The nucleolus of carcinoma cells assumed very peculiar forms (Text-Fig. 1). It might



Text-Figure 1. Appearance of nucleoli in malignant cells.

attain giant proportions up to the size of a small lymphocyte (6 μ) and occupy most of the nuclear mass (c). Nucleoli might be multiple, and were then usually irregular (a, d). The color might be light blue (a, b, c), as seen in noncancerous material, or dark grayish blue (d, e, f). Sometimes the outlines were indistinct and the nucleolar substance merged into the surrounding chromatin (f). At other times the nucleoli might be partially hidden in the mass of chromatin (giant cells, large round cells).

The *nucleus-cytoplasm ratio*, in my smear material, has often aroused the suspicion that carcinoma might be present, namely, when the mass of the nucleus was obviously larger in proportion to cytoplasmic mass than in the control cells. However, as already indicated, this cannot be considered a rule. The same may be said of the ratio of the nucleolar surface and/or diameter to that of the *nucleus*, which in carcinoma is usually much greater than in the control group. Zadek^{61,62} stated that the nucleolus-nucleus ratio is 1:4 to 1:20 in carcinoma cells in body fluids, compared to 1:25 to 1:100 in endothelial cells. MacCarty claimed that by his method the ratio is 1:5 to 1:17 in malignant processes, as against 1:13 to 1:45 in noncancerous processes. Since my method (smear) is different from that of Zadek (supravital stain) and of MacCarty (sections of fresh tissue), it is impossible to confirm or deny their conclusions.

Many of the changes mentioned above are seen also in histologic sections and are constantly utilized by pathologists. The smear technique permits better preservation of cytologic details and therefore more exact observation of minute structures. It must again be stressed that all of the features described above are suggestive, rather than diagnostic, of carcinoma. Extreme variations, however, may eventually prove to be diagnostic.

2. *Is there a cell, or are there cells, which characterize carcinomas, and are they always absent in noncancerous conditions?*

If malignant tumors express their abnormality not only in tissue structure (irregularity, infiltration), but also in cell structure, there arise two possibilities of recognizing cancer by its isolated cells: All of the cells of a tumor may have morphologic characteristics indicative of malignancy; during the development of a malignant tumor some of the cells may possess or acquire morphologic peculiarities as a consequence of abnormal function. It is these "peculiar" cells which would appear to be of importance in the recognition of cancer.

Smears of five different types, as previously described, were found in my material of 90 carcinomas, which were obtained from various organs and parts of the body and represented diverse histologic categories (adenocarcinoma, squamous cell carcinoma, comedo-carcinoma). A number of atypical cells were seen which were not found in smears of the control group,* consisting of 178 specimens from normal organs, inflamed tissues, and benign tumors. The atypical cells, listed below, were constantly present in the carcinoma smears of appropriate type:

(a) The nucleolated squamous cell (Figs. 7 and 8)

* Four exceptions to this statement are described in succeeding paragraphs.

- (b) The malignant giant cell (Fig. 13)
- (c) The malignant epithelial cell resembling basal cells (Figs. 9 and 10)
- (d) The pseudofibroblast (Figs. 11 and 12)
- (e) The malignant columnar cell (Fig. 16)
- (f) The undifferentiated cell (Fig. 19)
- (g) The large round cell (Figs. 17 and 18)
- (h) The oat cell (Fig. 5), only if in large sheets

These cells have been described above; the names have been selected on the basis of the most impressive feature of each. Cell *d* (pseudofibroblast) is not always distinguishable from fibroblasts; cell *h* (oat cell) is usually recognizable only when in sheets.

In some of the types of smears one of the above listed cells may predominate, or may be so numerous as to give the impression of being the only form present. This is usually the case in smears of the undifferentiated and round cell types. In other smears more than one kind of atypical cell was seen, as in the squamous cell type (nucleolated squamous cell, pseudofibroblast, giant cell), and in the columnar cell type (malignant columnar cell, large round cell, undifferentiated cell). Besides these atypical cells, in the majority of smears the proper cells of the organ in which the carcinoma was situated were seen also. In many cases it seemed possible to trace transitional forms from the atypical cells seen only in carcinoma smears to the epithelial cells of the control group. It should again be stressed that the atypical forms (*a* to *h*) are not considered to be the only malignant cells, since in smears of histologically proved metastases cells were seen which could not be distinguished from those found in inflammatory or other noncancerous processes. Another example of this observation is the smear of the oat cell type, in which often no cells were present which could not be found in the control group, and only the abnormal location and arrangement of the cells made probable the diagnosis of carcinoma. The assumption that a pathologic process may manifest itself in function only, without morphologic expression, would explain this finding.

Because previous workers^{2,4,61} frequently have sought a common denominator among the structures of cells obtained from malignant tumors, an attempt was made to find such factors in atypical cells (*a* to *h*) (size of nucleolus, giant vacuoles, ratio of nucleolus to nucleus). None had general application. The cells (*a* to *h*) as units differ from the cells of the control group, but not in their parts. The possibility may not be abandoned, however, that in some tumors one detail of the cell may be sufficiently constant and characteristic to

establish its diagnostic value. Quensel ⁴ and others ^{2,61} made such claims for the nucleolus of carcinoma cells in body fluids. The general application of such a rule does not seem to me to be justified.

For the 188 cases examined, there was agreement between the histologic diagnosis and the conclusion reached from the examination of smears in 179 cases. In the remaining 9 cases the conclusion reached from smears was apparently falsely positive in 5 instances and falsely negative in 4. These 9 cases are summarized in the following paragraphs.

A. Apparently False Positives

1. Case 33 was a 35-year-old female considered clinically to have a carcinoma (stage I) of the cervix. Histologic examination did not confirm the diagnosis, but showed a chronic cervicitis. The smear revealed a squamous cell carcinomatous type.

2. Case 37 was a 34-year-old female with a clinical diagnosis of menorrhagia. Sections removed from the corpus uteri showed progesterational endometrium; from the cervix, no lesions. Smears of the cervix revealed some nucleolated squamous cells with a more grayish violet cytoplasm than is usually seen in this cell form. No other cells of types *a* to *h* were seen.

3. Case 172 was a 66-year-old male with a clinical diagnosis of carcinoma of the face (temporal area). The pathologist reported: "Here and there in the dermis are isolated atypical cells with hyperchromatic nuclei that possibly are degenerating neoplastic elements, but of this we cannot be certain." The smear showed cells of characteristic squamous cell carcinoma type.

4. Case 185 was a 26-year-old female with a questionable clinical diagnosis of carcinoma of the breast. Sections taken for biopsy showed cystic hyperplasia of the breast; the smear showed the oat cell type.

5. Case 188 was a 20-year-old female with the clinical diagnosis of questionable benign tumor of the breast. Sections taken for biopsy showed lobular hyperplasia and fibrosing adenomatosis of the breast. The smears showed again the oat cell type and some giant cells, as illustrated in Figure 13.

B. Apparently False Negatives

1. Case 78 was a 46-year-old female. The clinical diagnosis was carcinoma of the breast; the histologic diagnosis was canalicular carcinoma of breast. The smear made from tissue in the neighborhood of the removed tumor could not be evaluated because of changes due to the antiseptic agent used in that area. Another smear taken from the periphery of the breast was negative for cells *a* to *h*.

2. Case 103 was a 45-year-old female with the questionable clinical diagnosis of carcinoma of the breast. The sections showed comedo-carcinoma of breast. The smear taken from the neighborhood of the tumor was negative for cells *a* to *h*.

3. Case 127 was a 43-year-old female. The clinical diagnosis was breast tumor; the histologic diagnosis, scirrhus carcinoma. The smear was negative.

4. Case 215 was a 57-year-old female. The clinical diagnosis was carcinoma of the breast; the histologic diagnosis, scirrhus carcinoma. The smear taken from the depth of the tumor showed only different forms of epithelial and connective tissue cells.

In the group of apparently false positive diagnoses, cases 33 and 172 presented the characteristic squamous cell type, despite the absence of carcinoma in the histologic preparations. I cannot explain this dis-

crepancy. Some investigators^{3,53} using Papanicolaou's method on vaginal smears have noted false positives similar to mine. The correctness of one or the other finding can be determined only by observation of the patient's future course. Cases 37, 185, and 188 showed only a few cells of types *a* to *h*.

In the second group of apparently false negative diagnoses, the failure to detect tumor cells in the smears from cases 78 and 103 is not significant, since the tissue was taken from the neighborhood of the tumor rather than from the tumor itself. Although the possibility of failure in these cases was realized, the smear was taken nevertheless, since in some cases tumor cells were detected far from the neoplastic center and from locations which the pathologist reported as containing "no lesion" (Fig. 20). Cases 127 and 215 must be considered failures. It is of interest that most instances of disagreement between histologic diagnosis and cytologic findings occurred in breast tissue (6 of 9). A possible reason, at least for the negative cytologic findings, is the large amount of fat present, which makes it more difficult to obtain satisfactory smears.

In summary, specimens from 268 regions were examined from the 188 cases. In 9 instances the cytologic and histologic findings were in apparent disagreement. One or another form of atypical cell (*a* to *h*) was found in the smears of 86 of 90 histologically recognized carcinomas. Usually more than one cell form appeared in the individual smear. Similar atypical cells have been found also in 4 cases classified histologically as noncancerous. In the smears of 174 of 178 specimens histologically classified as noncancerous (no lesion, inflammation, benign tumor) the search for cells *a* to *h* gave negative results.

3. *Is there any relationship between the organ from which a tumor arises and the type of carcinoma cells? That is, do the cells of a carcinoma of the breast differ from those of a carcinoma of the stomach or the skin?*

This material indicates that the organ within which a carcinoma arises exerts little influence upon the type of smear from that carcinoma. However, as has already been shown (Table III), smears of certain types are seen more frequently in certain organs than in others. This is probably dependent upon the type of epithelium which exists in that particular organ. Thus, the squamous cell type is met commonly in neoplasms of organs which normally possess squamous epithelium; the columnar cell type predominates in neoplasms of the mucosa of the gastro-intestinal tract. This is shown in Table V.

4. *Is there any relationship between the structure of a given carcinoma (adenocarcinoma, squamous cell carcinoma) and its cytologic*

character in smears? Are the cells which make possible the cytologic recognition of carcinoma the same in an adenocarcinoma as in a squamous cell carcinoma?

Table VI shows the relationship between the histologic structure of carcinomas and the types of smears obtained from them.

As may be seen, the type of the smear is related to the histologic

TABLE V
Relationship Between Site of Carcinoma and Type of Smear

Organ	Type of smear					No finding	Total
	Squamous cell	Columnar cell	Round cell	Undifferentiated cell	Oat cell		
Lip	3						3
Mouth	2						2
Esophagus	1						1
Stomach		4	3	2			9
Colon		5		1	1		7
Rectum		6		2			8
Larynx	1						1
Lung	3		1				4
Mediastinum			1				1
Uterus	5(2)*	3	2				10(2)
Ovary		2		3			5
Vulva	2						2
Breast			4	4	(2)	4	12(2)
Pancreas			1				1
Thyroid					1		1
Kidney	1						1
Bladder			1				1
Peritoneum				2			2
Neck	1						1
Lymph node	5	2	1		5		13
Abdominal wall				1			1
Chest wall				1			1
Skin	3						3
Total	27(2)	22	14	16	7(2)	4	90(4)

*Numbers in parentheses indicate negative histologic findings.

classification. All of the squamous cell carcinomas yielded smears of squamous cell type. The adenocarcinomas, however, are distributed among the columnar, round, undifferentiated, and oat cell types with predominance of the first three. The group classified histologically only as "carcinoma" produced smears of different types, since it embraced cases of carcinoma whose histologic appearance did not permit a more detailed classification. The special forms of breast tumors (duct carcinoma and canalicular carcinoma) fall into the round cell and undifferentiated types.

5. *Is the cytologic picture (type of smear) of a metastatic tumor the same as that of the primary tumor?*

It may be seen (Table VII) that of 17 examples of metastatic carci-

noma, 6 retained the original pattern in the metastases, 5 changed into the oat cell type of smear, whereas for the remaining 6 no conclusions could be drawn because the primary carcinoma was not available for study. As may be noted, when a change occurred in the metastases, it was always to the oat cell type, regardless of the type represented by the primary tumor. This change was seen only in metastases in lymph nodes. The results, although too few in number to justify conclusions,

TABLE VI
Relationship Between Histologic Diagnosis and Type of Smear

Histologic diagnosis	Type of smear					No finding	Total
	Squamous cell	Columnar cell	Round cell	Undifferentiated cell	Oat cell		
Squamous cell carcinoma	20						20
Adenocarcinoma		21	7	10	3		41
Adenoma, malignant		1					1
Carcinoma (without classification)	7		4	4	4		19
Duct cell carcinoma			1				1
Canalicular carcinoma			1			2	3
Medullary carcinoma			1	1			2
Scirrhus carcinoma				1		2	3
Benign	(2)*				(2)		(4)
Total	27(2)	22	14	16	7(2)	4	90(4)

*Numbers in parentheses indicate negative histologic findings.

TABLE VII
Comparison of Cytologic Findings in Primary Carcinomas and Their Metastases

Organ	Primary tumor		Metastases	
	Histologic diagnosis	Type of smear	Organ	Type of smear
Mouth	Squamous cell carcinoma		Lymph node	Squamous
Stomach	Adenocarcinoma	Undifferentiated	Lymph node	Oat cell
Stomach	Adenocarcinoma	Columnar	Lymph node	Columnar
Stomach	Adenocarcinoma	Round	Mediastinum	Round
Colon	Adenocarcinoma	Columnar	Lymph node	Columnar
Colon	Adenocarcinoma		Ovary	Columnar
Rectum	Adenocarcinoma	Columnar	Lymph node	Oat cell
Bronchus	Carcinoma	Squamous	Lymph node (1)	Oat cell
Bronchus	Carcinoma	Squamous	Lymph node (2)	Oat cell
Lung	Carcinoma	Round	Lymph node	Round
Ovary	Adenocarcinoma		Uterus	Columnar
Ovary	Adenocarcinoma	Undifferentiated	Peritoneum	Undifferentiated
Breast	Carcinoma	Undifferentiated	Lymph node	Oat cell
Skin	Carcinoma		Lymph node	Squamous
Neck	Squamous	Squamous	Lymph node	Squamous
Scalp	Squamous		Lymph node	Squamous

have been discussed because of the relative lack of attention given to this finding in the literature (Naidu¹³).

CONCLUSIONS

Ninety carcinomas were studied cytologically, utilizing both Wilson's and Papanicolaou's stains. The smears were made directly from the tumors and were compared to similar preparations from 178 non-cancerous tissues.

Eight cell types were recognized as characteristic of carcinoma and were found in 86 of the 90 histologically proved cancers. Similar cells were found in only 5 of the 178 noncancerous controls.

A larger number of cases must be studied before the validity of other cytologic expressions of carcinoma may be considered as proved.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 197

- FIG. 1. Case 148. Bronchogenic carcinoma; smear of squamous cell type. Wilson's stain. $\times 137$.
- FIG. 2. Case 52a. Adenocarcinoma of rectum; smear of columnar cell type. Wilson's stain. $\times 295$.
- FIG. 3. Case 21. Ductus cell carcinoma of mammary gland; smear of round cell type. Wilson's stain. $\times 137$.
- FIG. 4. Case 150. Adenocarcinoma of ovary; smear of undifferentiated cell type. Wilson's stain. $\times 295$.

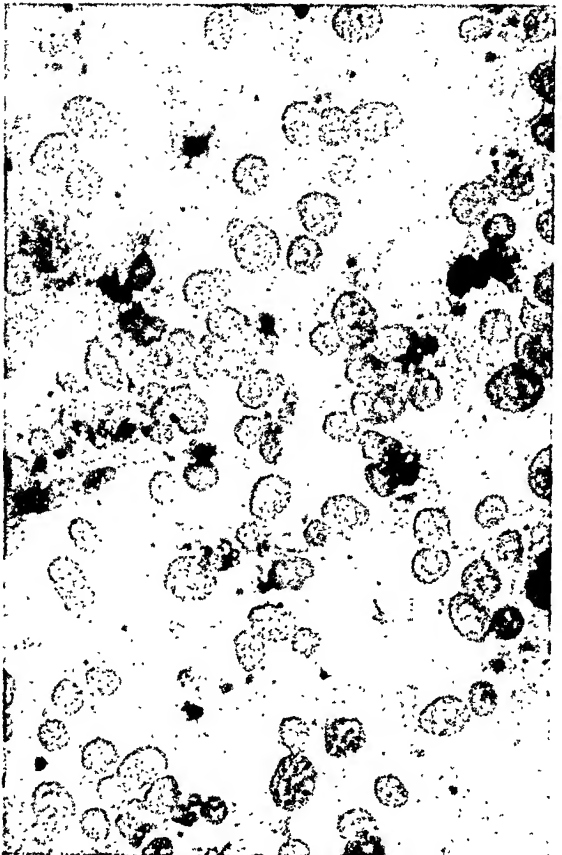
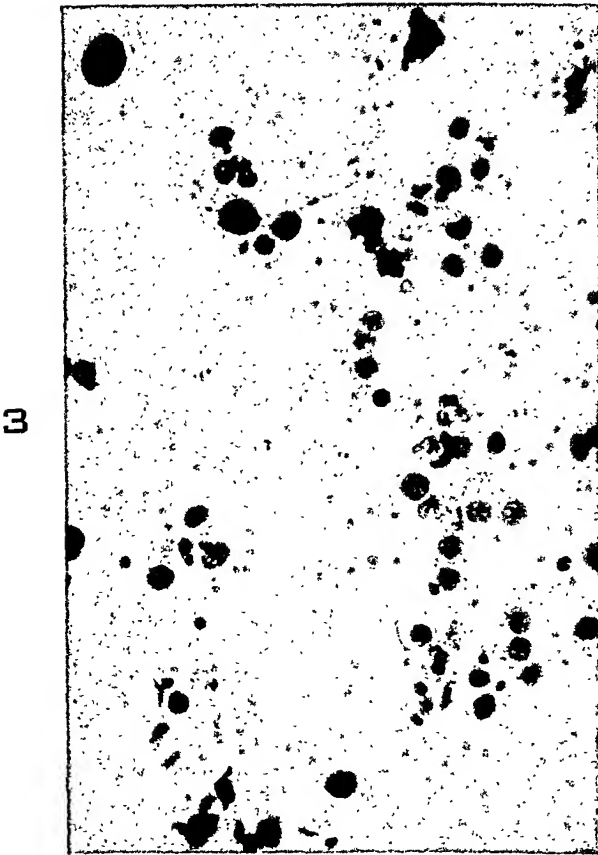
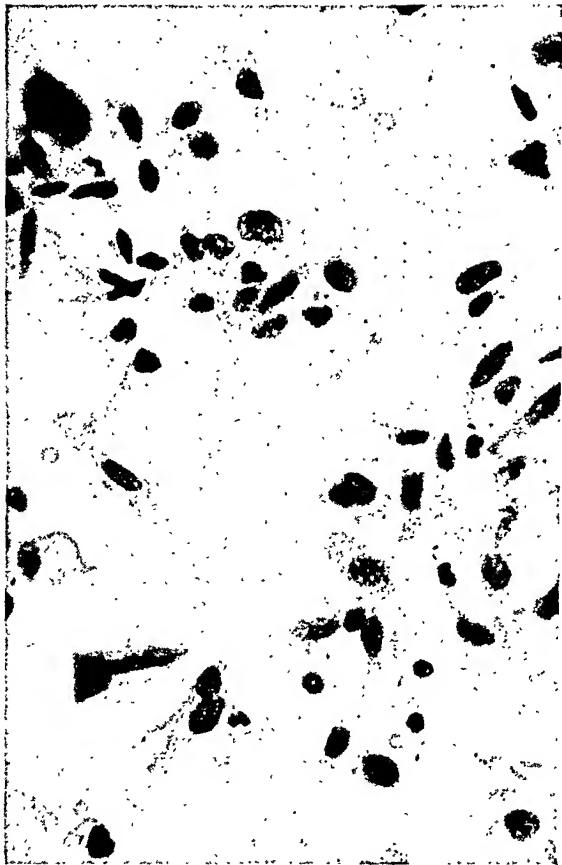
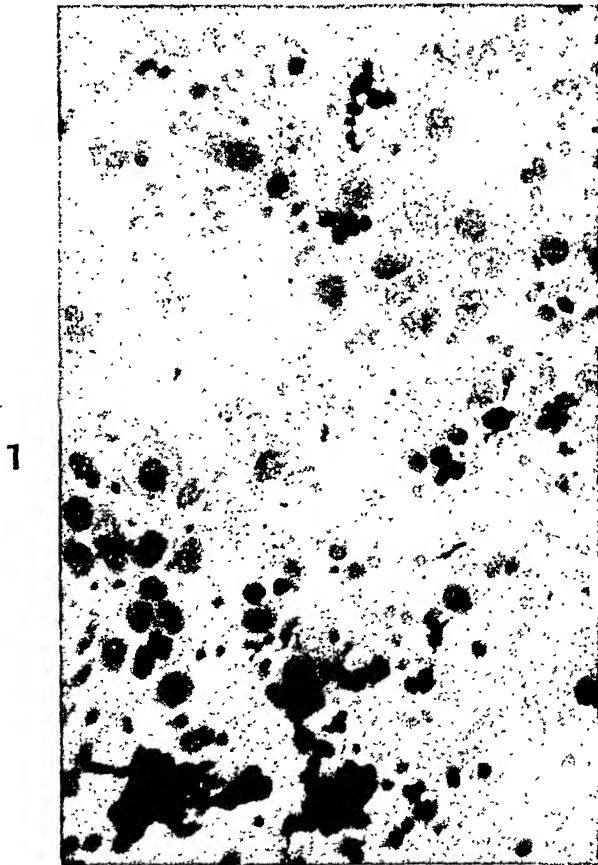
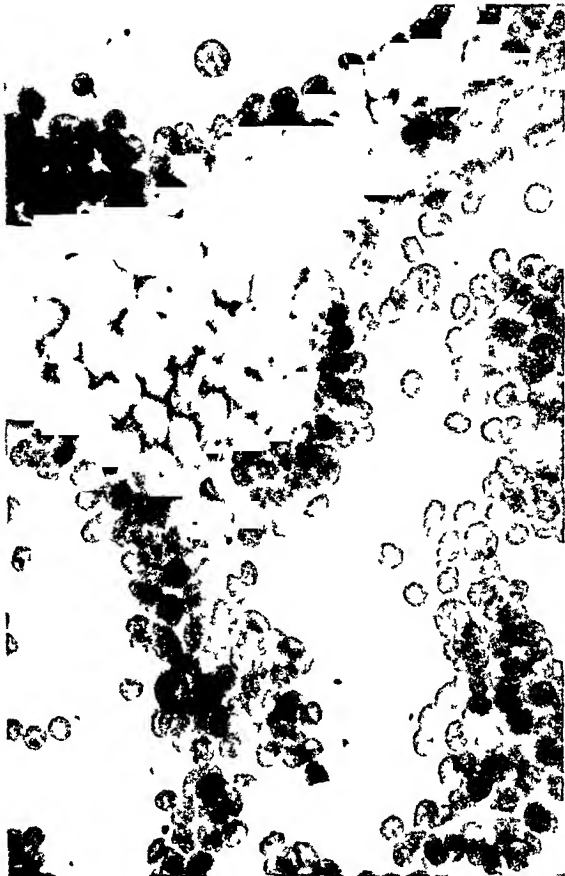


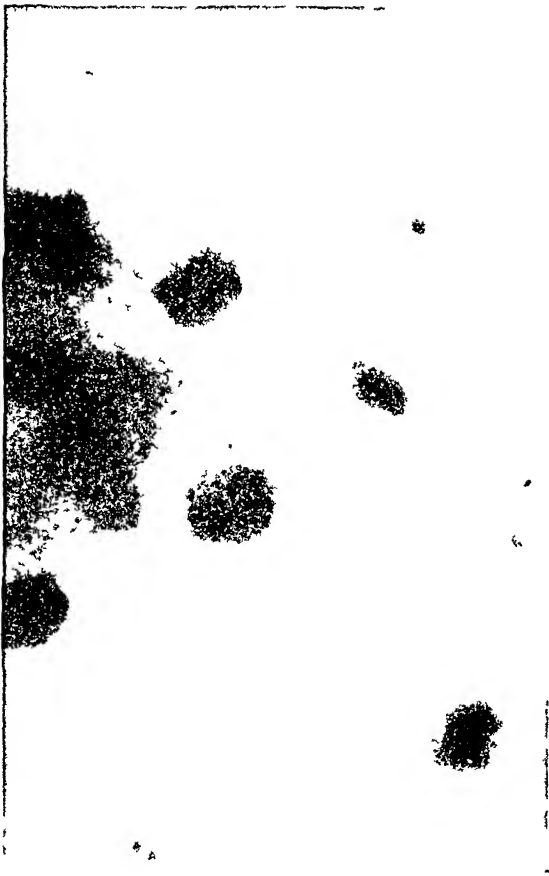
PLATE 198

- FIG. 5. Case 110c. Adenocarcinoma of thyroid; smear of oat cell type. Wilson's stain. $\times 295$.
- FIG. 6. Case 75a. Chronic cervicitis; squamous cells. Wilson's stain. $\times 684$.
- FIG. 7. Case 111a. Squamous cell carcinoma of cervix uteri; nucleolated squamous cells. Wilson's stain. $\times 684$.
- FIG. 8. Case 148. Bronchogenic carcinoma; nucleolated squamous cells. Wilson's stain. $\times 684$.

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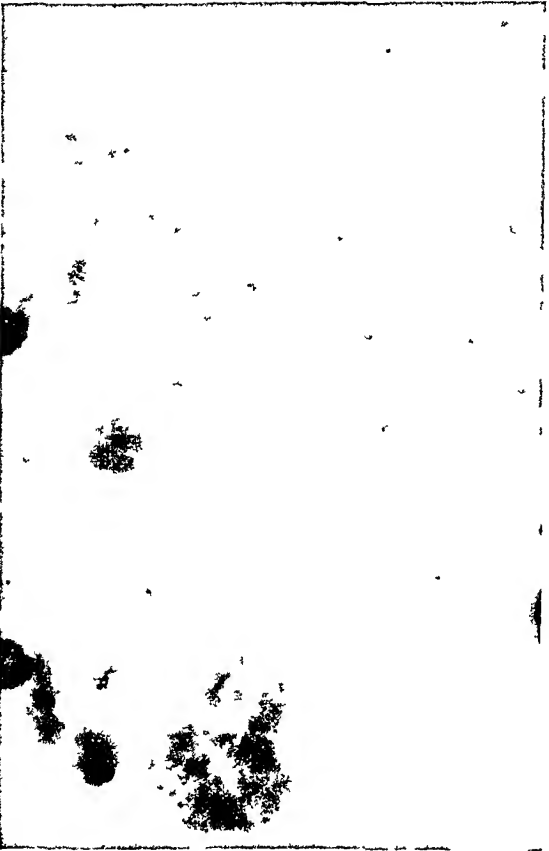
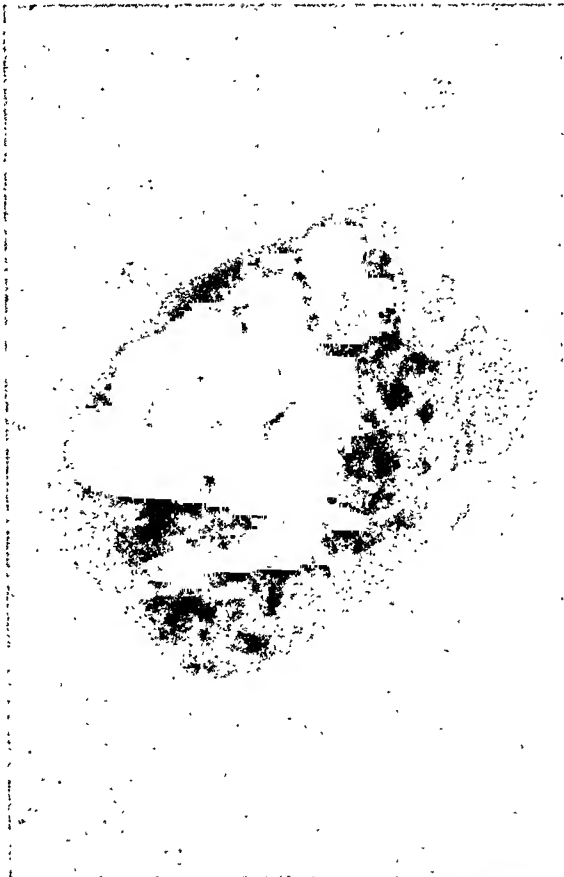


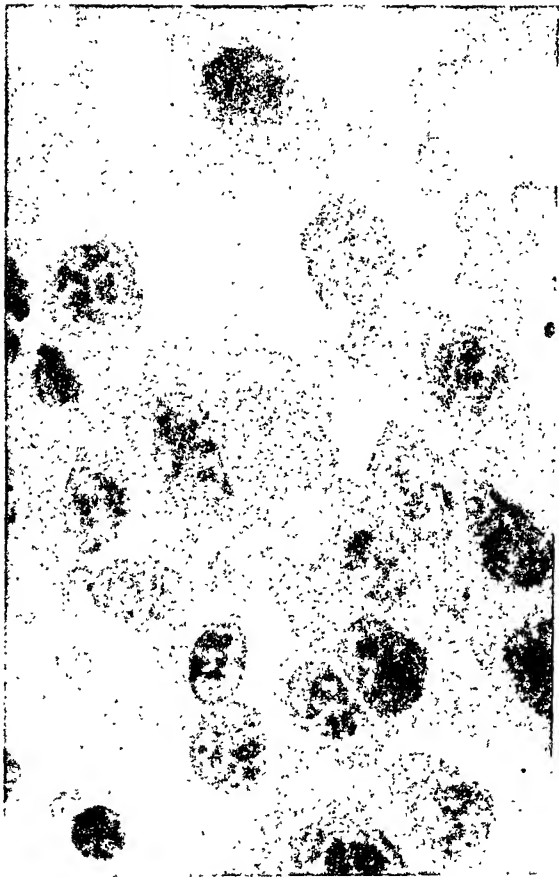
PLATE 199

- FIG. 9. Case 58. Squamous cell carcinoma of vulva; malignant epithelial cells. Wilson's stain. $\times 684$.
- FIG. 10. Case 11. Carcinoma *in situ* of cervix uteri; malignant epithelial cells. Wilson's stain. $\times 684$.
- FIG. 11. Case 158b. Squamous cell carcinoma of esophagus; pseudofibroblasts. Wilson's stain. $\times 295$.
- FIG. 12. Case 111a. Squamous cell carcinoma of cervix uteri; pseudofibroblast between two other cells, one in mitosis. Wilson's stain. $\times 684$.

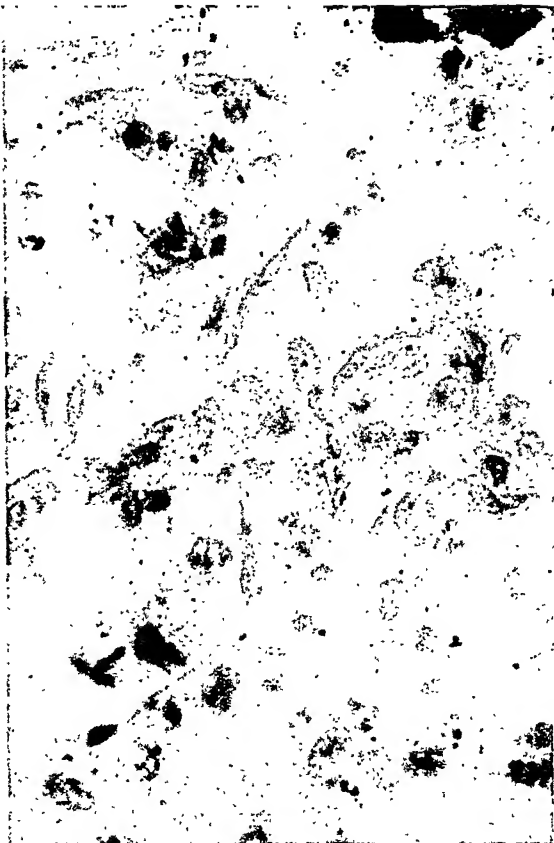
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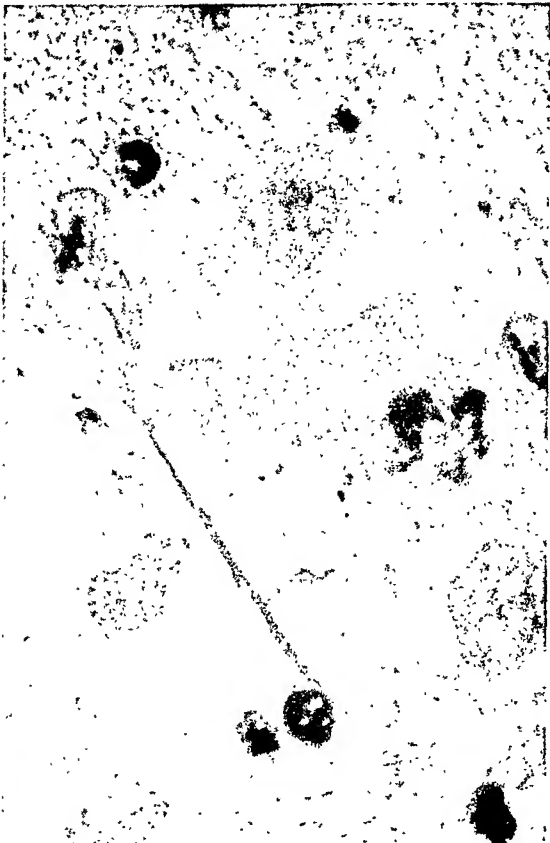


PLATE 200

FIG. 13. Case 119. Squamous cell carcinoma of lip; malignant giant cell. Wilson's stain. $\times 684$.

FIG. 14. Case 24a. Chronic granulomatous inflammation of lung; columnar cells from bronchial epithelium. Wilson's stain. $\times 684$.

FIG. 15. Case 140a. Chronic cervicitis and endocervicitis. columnar cells. Wilson's stain. $\times 684$.

FIG. 16. Case 52a. Adenocarcinoma of rectum; malignant columnar cells. Wilson's stain. $\times 684$.

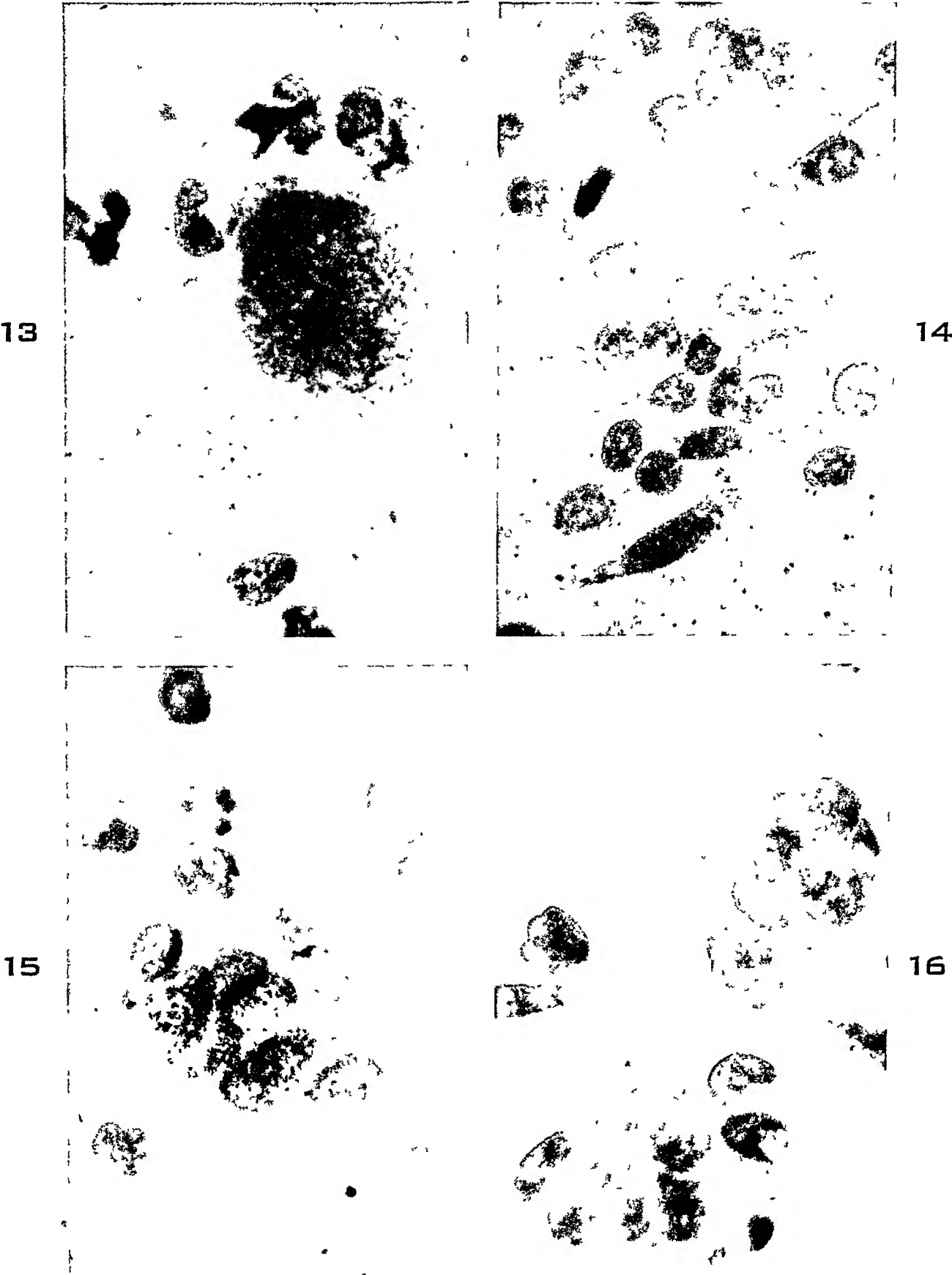


PLATE 201

FIG. 17. Case 21. Duct cell carcinoma of mammary gland; large round cells. Wilson's stain. $\times 684$.

FIG. 18. Case 14a. Adenocarcinoma of stomach; large round cells (giant form). Wilson's stain. $\times 684$.

FIG. 19. Case 150. Adenocarcinoma of ovary; undifferentiated cells. Wilson's stain. $\times 684$.

FIG. 20. Case 15b. Adenocarcinoma of stomach; tumor cells in fundic mucosa, histologically classified as "no lesion." Wilson's stain. $\times 350$.

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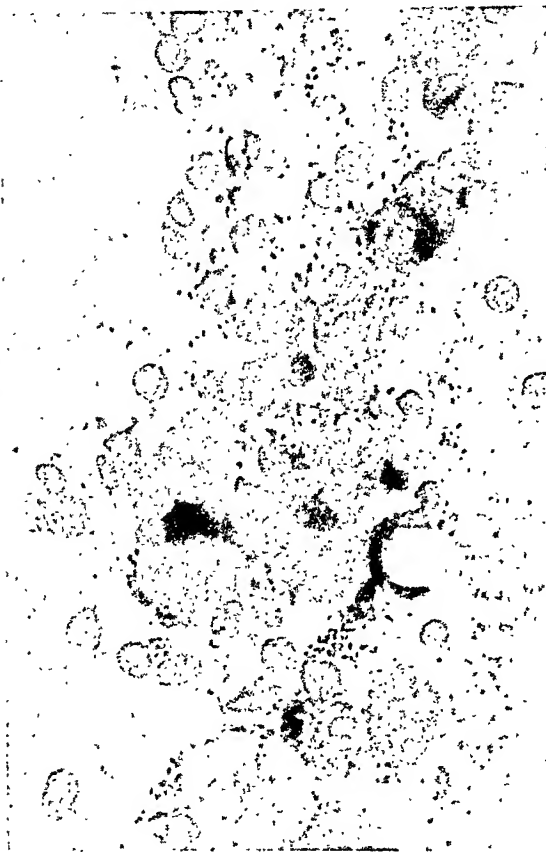
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DIFFUSE INTERSTITIAL MYOCARDITIS IN CHILDREN *

REGINALD K. HOUSE, M.D.†

(From the Department of Pathology, The Children's Hospital and The Infants' Hospital, Boston 15, Mass.)

In recent years there have appeared in the literature reports of a diffuse interstitial myocarditis of obscure etiology. What appears to be the same pathologic entity has been variously described as isolated, primary interstitial, circumscribed, diffuse, idiopathic, acute, subacute and chronic isolated, and has been termed myocarditis perniciosa, granulomatous myocarditis, and Fiedler's myocarditis.

No attempt will be made to review the literature, since Saphir and his associates¹⁻³ have done this thoroughly. It is difficult to arrive at any clear definition of this diffuse type of myocarditis, but it is generally agreed that it is a nonspecific inflammatory reaction of uncertain etiology. It is rarely diagnosed clinically, and most cases remain undiagnosed until the tissues are examined microscopically. The patient exhibits progressive myocardial failure, tachycardia, cardiac enlargement, cyanosis, and low blood pressure. The course tends to be rapid and death may ensue suddenly. At autopsy, the pathologist has little to aid him in making a gross diagnosis, but usually there is evidence of congestive cardiac failure. The heart is enlarged and dilated, and the myocardium tends to be flabby and paler than is normal. Microscopic examination reveals infiltration of the myocardium by lymphocytes, large mononuclear cells, polymorphonuclear leukocytes, eosinophils, and plasma cells without involvement of the pericardium or endocardium. An inflammatory lesion of this type is more frequently seen than others, although Saphir called attention to the granulomatous type, which has appeared in case reports. Neither the gross nor the microscopic findings explain the pathologic changes found in the heart, and it is usually this organ that shows the major lesion. It should be emphasized that the histologic picture is nonspecific and similar to that seen in the diffuse myocarditis associated with various infectious diseases. Yet, as Saphir stated in his review, one is justified in accepting the occurrence of isolated myocarditis in the sense of a more or less diffuse inflammatory lesion if every known cause for this type of myocarditis is ruled out and if there is present no major pathologic condition involving either the endocardium and pericardium or the entire body.

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† Now at Dartmouth Medical School, Hanover, N.H.

A recent autopsy of an infant performed at The Children's Hospital, Boston, revealed findings which were consistent with myocarditis of this type. The cases of myocarditis at this hospital thereupon were reviewed, and among them were found 4 which seemed to fit this classification. Since most instances of diffuse interstitial myocarditis of unknown etiology have been reported in adults, it has seemed appropriate to present the reports of these in infants.

REPORT OF CASES

Case 1

B. C., a 3-weeks-old girl, was admitted to the Infants' and Children's Hospital (no. 18940) on September 22, 1933, because of projectile vomiting, cyanosis, and dyspnea of 3 hours' duration. The family history was noncontributory. Delivery had been accomplished by high forceps. The birth weight was 10½ lb. The history of the first 3 weeks of life was uneventful. The patient had been breast-fed and was thought to be perfectly normal and healthy. Three hours before admission she awakened and vomited forcibly, and in 1½ hours she became very cyanotic, with labored respirations which continued until admission.

Physical examination revealed a well developed and well nourished female infant in extremis, with labored and rapid respirations and cyanosis. The anterior fontanelle was full but not tense, and the sutures were slightly separated. The pharynx was mildly injected. There was respiratory retraction of the costal margins. Cardiac dullness extended to the left anterior axillary border. The lungs were resonant and without râles. The abdomen was full, with the liver palpable down to the iliac crest and the spleen barely so. A roentgenogram of the chest showed marked enlargement of the heart and diffuse pulmonary congestion. The impression of the roentgenologist was congenital heart disease with passive pulmonary congestion.

The hemoglobin was 75 per cent; the red blood cell count, 4,900,000; and the white blood cell count, 32,000, with 61 per cent polymorphonuclear leukocytes, 38 per cent lymphocytes, and 1 per cent monocytes. Culture of the blood proved to be negative. Wassermann and Hinton tests of the blood of both parents gave negative reactions. A spinal fluid reaction was negative.

The infant failed to respond to therapy and died 7 hours after admission. The clinical diagnosis was congenital malformation of the heart with cardiac decompensation.

Autopsy Findings

Autopsy, performed 12 hours after death, revealed a well developed and well nourished infant with marked lividity. The abdomen was slightly protuberant and markedly tense. The peritoneal cavity contained a considerable amount of clear, yellow fluid. The liver extended 4.5 cm. below the costal margin in the midline and the spleen was just visible on the left. Each pleural cavity contained 4 cc. of clear, straw-colored fluid. The heart and lungs together weighed 130 gm. The heart was increased in size. On the anterior surface of the left ventricle and directly in the path of the descending ramus of the left coronary artery was a slightly depressed yellow-gray area of softening which measured 8 by 5 mm. There were two similar but smaller,

soft, yellow-gray areas near the left border of the left ventricle. The muscle of the apex of the left ventricle was thin. Throughout the course of both coronary arteries, the periarterial tissue was slightly raised and moderately firm. At a point 1.4 cm. above the area of softening and extending for a distance of 0.6 cm. there was a loss of pericorony prominence, which gave an appearance of loss of contour of the artery. No congenital abnormalities were observed during complete dissection. The areas of softening in the myocardium were yellow-gray, but they did not appear to involve the pericardium or endocardium. The rest of the myocardium was slightly increased in opacity, dark pink and gray. Near the two small areas of softening there was observed a round, firm lesion, 1 mm. in diameter, which was thought by the examiner to be a thrombosed vessel. Both coronary arteries had slight tortuosity. The lungs were not remarkable except that on sectioning there was evidence of bronchopneumonia. The liver and spleen were congested and weighed 146 and 16 gm. respectively, somewhat overweight for the age. The brain was not remarkable. From cultures taken from the lung at the time of autopsy there were grown *Staphylococcus aureus*, *Escherichia coli*, and pneumococcus. There was no growth from the heart's blood. The impression from the gross findings was early bronchopneumonia and an enlarged heart of unknown cause.

Microscopic Examination. Tissues were fixed in Zenker's solution and in formalin. Multiple sections of the heart were stained with hematoxylin and eosin, Giemsa's, Gram's, Foot's reticulum, Levaditi's, and Ziehl-Neelsen stains. Sections taken through the softened areas mentioned in the gross description revealed widespread and diffuse absence of cardiac musculature with a moderate infiltration by polymorphonuclear leukocytes, lymphocytes, plasma cells, macrophages, and large mononuclear cells. These cells had large, vesicular, oval nuclei and scanty eosinophilic cytoplasm. The majority of the muscle fibers had been replaced by young granulomatous tissue. Although there were fibroblasts present, newly ingrowing capillaries were the prominent feature. In this region remnants of cardiac muscle, without striations, and vacuolated and poorly stained, were observed. There was extravasation of red cells in many fields. The lesion was not circumscribed, but it was irregular and penetrated into areas of normal appearing muscle fibers. For the most part, the muscle tissue immediately adjacent to the inflammatory reaction showed loss of striations, vacuolation, and an occasional pyknotic nucleus. Here there was moderate interstitial infiltration by similar inflammatory cells. In one section the lesion approached the endocardium, but there was minimal

exudation. There was no definite evidence of pericarditis, although the inflammatory reaction penetrated to the pericardium. On the epicardium there was no recognizable fibrin deposition or exudate. The blood vessels throughout were dilated and congested, but there was no suggestion of arteritis. The auricular musculature in one section showed interstitial edema, but there was no interstitial lesion. Sections of the main coronary arteries did not exhibit abnormal histologic findings. Foot's reticulum stain showed no increase in the stroma in the area of greatest damage. Gram's and Giemsa's stains failed to reveal any bacteria or inclusion bodies. Ziehl-Neelsen's and Levaditi's stains were also negative. Tissue was not available for fat stains.

The microscopic sections of the other tissues were noncontributory except for moderate interstitial pneumonia, toxic splenitis, chronic passive congestion, and slight fatty change of the liver.

Case 2

L. R., a 13-months-old girl, was admitted on February 24, 1941 (no. 24543), with persistent listlessness, drowsiness, anorexia, and dyspnea of 3 weeks' duration. The family history was irrelevant except that the father was under treatment for leukemia. This was the mother's first pregnancy and it had been uneventful. The birth weight was 5 lbs., 14 oz. The baby was breast-fed for 4 days and then transferred to formula because the mother had developed pneumonia. The infant was well and very active until 5 weeks before admission, when she developed an upper respiratory infection, which apparently cleared up within a few days. The medication consisted of oily nose drops. Three weeks before admission the patient had difficulty in breathing, refused her feedings, and became listless. The family physician found no elevation of temperature and made a diagnosis of bronchitis. The patient continued to do poorly and the breathing became progressively worse, but the mother found that the baby was more comfortable when sitting up. On repeated visits by the family physician, the temperature was never elevated.

The physical examination revealed an acutely ill but well developed and well nourished female infant. The temperature was 100.6° F.; pulse, 160; respirations, 84. The pharynx and tonsils were red. There were a few enlarged cervical lymph nodes. There was dullness over the right upper chest anteriorly and in the right interscapular region. Coarse rhonchi were heard over the entire chest, with many râles in the right lower lobe and the right axilla. The borders of the heart could not be made out. There was a definite gallop rhythm, and the heart sounds were of poor quality; no murmurs were heard. The liver and spleen were palpable 2 fingerbreadths and 1 fingerbreadth below the costal margin, respectively. There were no other masses or tenderness. Roentgenograms of the chest revealed a markedly enlarged heart and fine mottling of both lung fields. The radiologic report was cardiac dilatation and pulmonary edema, consistent with circulatory collapse. The hemoglobin was 50 per cent and the white blood cell count 13,000, with 50 per cent polymorphonuclear leukocytes.

On the patient's return to the ward following roentgenologic examination of the chest, she became dyspneic, and despite artificial respiration and stimulants she died 2 hours and 5 minutes after admission. The clinical diagnosis was pneumonia with cardiac failure.

Autopsy Findings

Autopsy, performed 2 hours after death, was restricted to the thorax and abdomen. The body was well developed and well nourished.

The right and left pleural cavities contained 32 and 50 cc. of clear, yellow fluid, respectively. The liver edge extended 5 cm. below the xiphoid process and the spleen 2 cm. below the left costal margin. The heart weighed 90 gm., as compared with a normal weight for this age of 44 gm. The myocardium was pale red-brown and flabby. There were no congenital abnormalities. The weight of both lungs was 257 gm., compared with the normal weight for this age of 121 gm. Both were congested, with increased consistency and decreased crepitation. The cut surface revealed bright pink tissue with large amounts of frothy pink fluid exuding. In some areas it exhibited a meaty, red appearance. The spleen and liver were both slightly enlarged, and there was gross evidence of fatty change of the liver. No other important gross findings were in evidence. Cultures of the heart's blood and fluid from the thoracic cavity were negative, but in cultures of the right lung was grown a type 19 pneumococcus. The gross findings were pneumonitis and hypertrophy of the heart, the cause of which was obscure.

Microscopic Examination. Tissues were fixed in Zenker's solution and in formalin. Sections of the heart were stained with hematoxylin and eosin, Giemsa's, Gram's, and Foot's reticulum stains, and scharlach R. The sections of the left ventricle revealed a diffuse interstitial infiltration by lymphocytes, predominantly, but there were occasional plasma cells, polymorphonuclear leukocytes, a rare eosinophil, and large mononuclear cells with large vesicular nuclei and abundant eosinophilic cytoplasm. Although the exudation was widespread in the left ventricle, it was not uniform throughout. There were many focal collections, and in some areas these were closely packed, particularly adjacent to the pericardium, where they formed groups in which the continuity of the muscle fibers was interrupted. There was a minimal involvement of the pericardium, with foci and scattered exudative cells beneath this layer. There was no inflammatory reaction on the epicardial surface. The endocardium was involved also in that portion contiguous with the myocardium, but not to a marked degree. The endocardium of the left auricle was thickened by an increase in the fibrous tissue. It was also edematous, but the endothelium was intact and without exudation. A section of a valve leaflet revealed some separation and fragmentation of the connective tissue, but there was no evidence of thickening, vascularization, or cellular

infiltration. The cardiac musculature exhibited little definite evidence of degeneration. The myocardial fibers were well preserved and without loss of striations, and only in a few areas were there occasional muscle fibers with fragmentation and shrinking, and distorted pyknotic nuclei. There was, however, a diffuse interstitial edema. The process in the left auricle was similar to that in the left ventricle but was much less severe. There were small focal collections of lymphocytes. The right ventricle also presented the same histologic picture as the left auricle. There appeared to be no involvement of the vascular system of the myocardium; neither was there any evidence of Aschoff bodies. Foot's reticulum stain was noncontributory, nor did Gram's or Giemsa's stains demonstrate bacteria or inclusion bodies. The scharlach R stain showed no demonstrable increase in stainable fat.

The other microscopic findings were congestion and edema of the lungs, a mild interstitial pneumonia, toxic splenitis, toxic adenitis, and fatty change of the liver.

Case 3

D. C., an 11-months-old girl, entered the hospital for the second time on August 28, 1946 (no. A-301221), with cough and fever of 2 weeks' duration. The family history was noncontributory. The patient had two older siblings living and well. The mother's pregnancy had been uneventful except for edema of the ankles, and the delivery had been normal. The neonatal course was without complication until at 2½ months of age, when the patient developed an upper respiratory infection followed at 3 months by diarrhea. At this time she was first admitted to the hospital. She was placed on penicillin and chemotherapy, but in spite of this the diarrhea persisted for the first few weeks. The temperature ranged from normal to 103°F. Twenty-three days after admission, bilateral mastoiditis was demonstrated roentgenologically. Bilateral antrotomy was performed, but the patient showed little improvement until 1 week after the operation, when improvement was rapid. She was discharged home on February 3, 1946, 31 days after admission.

The patient was apparently well until 2 weeks before the second admission, when she developed a cough, fever, and grunting respirations. Four days before admission she received her last injection of whooping cough vaccine, and a mixture of diphtheria and tetanus toxoids.

The physical examination revealed an acutely ill infant with respirations of 84 and a temperature of 103°F. She became cyanotic on crying. The chest revealed slight inspiratory retraction, hyperresonance, and expiratory wheezes. The liver was palpable but there were no other abdominal masses. Fluoroscopy revealed an enlarged heart and poorly aerated lungs. Roentgenologic examination of the chest on the first admission had shown the heart to be of normal size. The white blood cell count was 15,000, with 60 per cent lymphocytes and 28 per cent polymorphonuclear leukocytes. The infant failed rapidly in spite of oxygen, steam, penicillin, sulfadiazine, and aminophylline and died 15 hours after admission. The clinical diagnosis was acute bronchiolitis.

Autopsy Findings

Autopsy, performed 6 hours after death, revealed a well developed and nourished female infant with slight pitting edema of the feet and

cyanosis of the lips. Examination was restricted to the thorax and abdomen. The liver edge was 4 cm. below the xiphoid process. There was no fluid in either pleural cavity. The heart was enlarged and dilated. The myocardium seemed normal except for the region of the interventricular septum, where there was some gray streaking. The endocardium was smooth and glistening except for moderate opacity and thickening in the region of the septum. The coronary arteries were injected by the technic of Schlesinger⁴ and no anomalies or defects were found. The lungs exhibited some atelectasis and emphysema. There was evidence to support the diagnosis of aspiration pneumonia. The abdominal viscera were congested and edematous. In cultures of the heart's blood *Escherichia coli* was grown, but the lung cultures were negative. The gross findings were consistent with congestive cardiac failure. The cause for cardiac hypertrophy was not established.

Microscopic Examination. Tissues were fixed in Zenker's solution and in formalin. Multiple sections of the heart were stained with hematoxylin and eosin, Giemsa's, Gram's, Foot's reticulum, and Weigert's elastic tissue stains, and scharlach R. All sections of the left ventricle were histologically similar. They presented a diffuse interstitial infiltration by lymphocytes with occasional polymorphonuclear leukocytes, plasma cells, and a rare eosinophil. The exudation was not uniform and in many areas exhibited large, ovoid collections of lymphocytes. In these particular areas the muscle fibers had lost their continuity and were not recognizable as such. In the remaining myocardium there was little evidence of damage except in one area, where there was a fine interstitial fibrosis adjacent to the endocardium. There was an occasional muscle fiber which showed hyaline degeneration, but the majority appeared histologically normal. There was, however, a diffuse, moderate, interstitial edema. The epicardium was not involved except that the exudate seemed to be more abundant in the myocardium adjacent to it. Nowhere was there evidence of pericarditis. The left ventricular endocardium was thickened by fibrous tissue, and there was a slight infiltration of this tissue that was contiguous with the myocardium. The interventricular myocardium presented an identical picture with an abundant lymphocytic exudate and edema that was more severe than that seen in the left ventricle. Sections of the right ventricle and left auricle also showed a similar interstitial exudation. Here the focal collections and scattered lymphocytes were much less abundant. Sections of the right auricle presented only a minimal inflammatory reaction. There appeared to be no abnormality of the coronary arteries. Sections of the mitral, aortic, and tricuspid valves showed no lesions except for slight edema. Weigert's stain

revealed increased elastic tissue in the left ventricular endocardium. Foot's reticulum stain showed no apparent increase in reticulum except for one section of the left ventricle, which revealed an increase in fibrous tissue of the myocardium, near the endocardium. Giemsa's and Gram's stains demonstrated no evidence of bacteria or inclusion bodies. Scharlach R stain revealed no increase in stainable fat. The other microscopic findings were a mild interstitial pneumonia, an aspiration pneumonia, edema and congestion of the lungs, lymphoid hyperplasia, and fatty change of the liver.

Case 4

R. D., a 12-months-old boy, was admitted on October 15, 1946 (no. A-46-235). He was in good health until 2 weeks before admission, when suddenly, while sitting in a chair, he began to scream as if in pain, developed pallor, and sweated profusely. This persisted for 15 minutes and subsided as abruptly as it had begun. Following this episode the patient was irritable, ate poorly, and sat up in order to sleep. In the 2 weeks preceding admission there were three or four attacks of a similar nature, lasting the same length of time, and with some of them there was retching but no vomiting. They had no particular relationship to activity or feeding. There was no fever, but a gradual increase in dyspnea, malaise, anorexia, and some blueness of the lips were noted.

Physical examination revealed an acutely ill infant, well nourished and well developed. The temperature was 100.8°F.; pulse, 180; respirations, 78. The blood pressure was 90/65 mm. of Hg. The skin was moist and gray-white. No edema was present. There were fine inspiratory râles over both lung bases. The heart was enlarged to percussion and the sounds were of poor quality. The rate was rapid but the rhythm was regular. The liver edge was felt at the iliac crest and the spleen was just palpable. Roentgenologic examination of the chest showed the heart to be markedly enlarged to the right and left, with considerable enlargement of the pulmonary vessels and congestion and edema of the lung fields.

The electrocardiogram showed sinus tachycardia, right axis deviation, and low T waves in all leads. Examination of the urine showed 0 to 1 plus albumin. The hemoglobin ranged from 9.6 to 13.1 gm.; red blood cells, 4.0 to 4.75 millions; white blood cells, 7,100 to 16,500, with 5 to 54 per cent polymorphonuclear leukocytes, 36 to 94 per cent lymphocytes, 8 to 1 per cent monocytes, and 1 per cent eosinophils. No pathogens grew in a culture from the nose. A glucose-tolerance test and an adrenalin-tolerance test gave normal reactions.

The patient improved under treatment with oxygen, digitalization, thiocalcin by rectum, and sedation. The pulse dropped to 108 and the liver edge was palpable at the umbilicus. Repeated roentgenograms of the chest showed clearing of the lungs and a gradual decrease in size of the cardiac silhouette. The patient did well and was discharged home on the 24th hospital day, although the pulse remained above 100. On the day after discharge he developed an upper respiratory infection, followed in 24 hours by anorexia, dyspnea, and an ashen-gray color. He was readmitted on the 3rd day after discharge. The findings were similar to those of the first admission, and similar therapy was instituted with the addition of chemotherapy. However, chemotherapy was stopped within 2 days because of a decrease in the white blood cell count and a marked shift to lymphocytes. The second electrocardiogram showed sinus tachycardia, right axis deviation, low T-1, diphasic T-2, and inverted T-3. Aside from nasopharyngitis, the patient did well and was discharged to a convalescent home 3½ weeks after the second admission. Within

24 hours after discharge he developed fever, cough, dyspnea, and restlessness, and was readmitted in a moribund state. He died 2 hours after admission, approximately 66 days after the onset of illness. The clinical diagnoses were myocarditis, pneumonia, and congestive cardiac failure.

Autopsy Findings

Autopsy was performed 12 hours after death. Each pleural cavity contained 50 cc. of clear, yellow fluid, while the pericardial sac contained 20 cc. The heart was dilated and weighed 111 gm., as compared with a normal weight of 45 gm. The pericardium was smooth and glistening and presented several small petechiae. The myocardium was soft, flabby, and red-brown, and in some areas was paler than is normal. The endocardium of the left ventricle was opaque and on section the surface appeared to be slightly thickened. No congenital abnormalities were seen. The lungs were increased in weight, congested, and edematous. There was some gross evidence of pneumonitis. There was congestion of the abdominal viscera with enlargement of both the liver and the spleen. The brain was edematous and congested. The findings were consistent with congestive cardiac failure. A post-mortem blood culture yielded no growth, while from the lung *alpha* streptococcus and *Micrococcus catarrhalis* were grown. The cause for hypertrophy of the heart was not found.

Microscopic Examination. Tissues were fixed in Zenker's solution and in formalin. Sections of the heart were stained with hematoxylin and eosin, Gram's, Giemsa's, Foot's reticulum, Weigert's elastic, and scharlach R stains. Sections of the left ventricle revealed an interstitial infiltration by lymphocytes, polymorphonuclear leukocytes, plasma cells, and eosinophils. The predominant cells were lymphocytic. The infiltrate was not uniform throughout the sections. In some, it was abundant with many focal collections, and in areas it appeared to be more prominent in the vicinity of the pericardium. Other sections showed minimal involvement. The moderate amount of subepicardial fat showed some cellular infiltration, but nowhere did there appear to be any recognizable pericarditis. Many of the exudative cells were in the vicinity of the coronary arteries, but there was no evidence of a vascular lesion. There was moderate thickening of the left ventricular endocardium by fibrous tissue, but here again there was minimal involvement of the endocardium by inflammatory cells in areas contiguous with the myocardium. There was moderate interstitial edema of the myocardium, but there was little evidence of degeneration. Only in the focal areas of lymphocytes were the myocardial fibers absent. In general, the cardiac muscle was well preserved and only occasionally was there a pyknotic nucleus. There was moderate con-

gestion of all blood vessels. The right ventricle presented similar interstitial infiltration, predominantly by lymphocytes. The exudate was spotty and much less severe than that of the left ventricle. Here again it was more abundant near the epicardium. Sections of the left and right auricle showed only an occasional focus of lymphocytes in close proximity to small arterioles; the Weigert stain exhibited a slight increase in elastic fibers in the left ventricular myocardium. Giemsa's and Gram's stains were negative for bacterial inclusion bodies. Foot's reticulum stain showed no increase in connective tissue. Glycogen and scharlach R stains gave only negative results. Sections of the mitral valve revealed no evidence of abnormal structure.

Other microscopic findings were a terminal bronchopneumonia, moderate congestion of liver and spleen, and toxic splenitis.

DISCUSSION

It is not the primary purpose of this paper to discuss the clinical aspects of these cases, but there were striking similarities in all four. The onset of symptoms was relatively of short duration, ranging from 3 hours to 3 weeks. Except for case 3, the mothers had observed cyanosis, dyspnea, and anorexia. The family and past histories were all essentially noncontributory except for case 3, and these factors will be discussed in relation to possible etiologic agents. On admission to the hospital the patients were acutely ill, with rapid pulse, labored respirations, and an elevated temperature. There was enlargement of the heart either by physical examination or roentgenologically. Several electrocardiograms were done in case 4 and all showed sinus tachycardia, right axis deviation, and low T-waves in all leads, which suggested myocarditis or a digitalis effect. The lungs presented positive findings by percussion and auscultation, except for case 1. The liver and spleen were palpable in all cases except case 3, in which only the liver was felt. The course in the hospital was of short duration in the first 3 cases, and was measured in hours. Only in case 4 did the patient survive sufficiently long for an adequate clinical study. The pathologic findings were essentially the same. The bodies were well developed and well nourished. The heart was enlarged and dilated, and an adequate cause for hypertrophy was not demonstrated at the time of the autopsy. Congenital abnormalities were lacking. The coronary circulation followed the usual anatomic pattern, although in case 1 the prosector believed that he was dealing with a possible coronary occlusion. Microscopic sections of the coronary arteries, however, did not substantiate the gross impression. None of the types of coronary occlusion described by Stryker⁵ were found. The myocardial lesions

of case 1 did not resemble either tuberculosis or syphilis. Neither did this case resemble the characteristic granulomatous lesion described by Saphir,² Magner,⁶ and Jonas.⁷ This form of myocarditis is a clearly defined one, not unlike known granulomas and sometimes confused with syphilis and tuberculosis. It may well be that the heart of case 1 is an example of a myocarditis developing in utero. The remaining cases were all histologically similar and varied only in the amount of exudate. In summarizing the microscopic picture, the myocardium was diffusely involved by an interstitial exudate, consisting primarily of lymphocytes, but with an occasional polymorphonuclear leukocyte, plasma cell, eosinophil, and large mononuclear cell. There was moderate to severe interstitial edema, but there was little evidence of myocardial damage. Nowhere was there a specific lesion found, such as the Aschoff body. The endocardium and epicardium showed only minimal involvement. In cases 3 and 4 there was endocardial fibrosis, which was interpreted as fibro-elastosis (fetal endomyocarditis). The ventricular endocardium was moderately thickened by elastic fibers with extension into the underlying myocardium. In only one case (no. 3) was there patchy fibrosis of the myocardium. The lungs of all cases showed gross and microscopic evidence of interstitial pneumonia or bronchopneumonia. This was a terminal complication and histologically the inflammatory lesions of the heart antedated the pulmonary process. It is important to point out that no other major pathologic lesion was found. The incidental findings, such as the congested abdominal viscera, could all be explained on the basis of congestive cardiac failure. These 3 cases (2, 3, and 4), from the clinical and necropsy observations, appear to fall into the second more common type of diffuse interstitial myocarditis.

The etiologic basis for myocarditis of this type is unknown. The multiplicity of agents suggested in the literature makes this evident. For the granulomatous type it has been stated that tuberculosis or syphilis may be a possible cause even though the causative organisms are not demonstrated. Baker and Brian⁸ have called attention to blastomycetes as producing granulomas in the heart, and it appears possible that other organisms could produce this same type. Lillie and Francis⁹ have shown that *Pasteurella tularensis* can produce granulomas in the hearts of experimental animals.

The diffuse form of interstitial myocarditis has appeared more frequently in the literature, and as a result more agents have been suggested as possible factors in causing this pathologic process. Severe burns, upper respiratory infections, toxins, influenza, and infectious diseases have been mentioned. According to other case reports, the

administration of adrenalin has produced myocarditis of a similar type. Experimentally, it has been produced with sparteine and adrenalin combined. Bismuth and the arsenicals have been attributed as factors in producing this condition. In relation to this possible allergic response, French and Weller¹⁰ reported 126 cases of interstitial myocarditis in which sulfonamide therapy was thought to be the common etiologic agent. Wells and Sax¹¹ recently have reported a case in which they believe sulfadiazine might well have been the cause of a myocarditis. Vitamin deficiencies, idiosyncrasies to sulfur ointment, and alcohol have been mentioned. Helwig and Schmidt,¹² in 1945, reported the death of two chimpanzees in their laboratory from cardiac failure and pulmonary edema. Histologically, the principal finding was an acute interstitial myocarditis. Pleural fluid from one animal was passed through 122 mice and with rare exceptions produced paralysis and acute myelitis. This same material has been used to produce similar myocarditis in guinea-pigs and rabbits. Finland, Parker, Barnes, and Joliffe¹³ have reported two cases of influenza A pneumonia with a nonbacterial myocarditis. This would suggest that a virus infection may be linked with myocarditis of this type.

In reviewing the possible etiologic factors reported in the literature, it would seem that the majority of them could be eliminated in the 4 cases reported here, particularly in this age group.

In case 1, the cardiac lesion of this 21-day-old infant was nonspecific, although it suggested more of a granulomatous process. Histologically, it did not resemble a gumma or tuberculosis. Levaditi's stain and acid-fast stains were both negative. Serologic examination of both parents was negative. Another possible factor which must be considered is a coronary occlusion, even though a careful examination of the heart was made grossly and microscopically. At this relatively early age an infectious process developing in utero must be thought of also.

In case 2 there was the history of the mother developing pneumonia on the fourth day post-partum, and for that reason the baby was removed from breast feedings. This infant also contracted an upper respiratory infection 5 weeks before the onset of the terminal illness.

In case 3 there was an upper respiratory infection at 3 months of age followed by bilateral otitis media. At this time chemotherapy was used and it was also administered during the brief last illness. Four days before the last admission she received the last injection of whooping cough vaccine, tetanus and diphtheria toxoid. On admission to the hospital a diagnosis of an upper respiratory infection was made. One might possibly conceive that the myocarditis in these two cases (2 and 3) was produced on an allergic basis since both gave a history of recent and

former upper respiratory tract infections. Mallory and Keefer¹⁴ have observed focal accumulations of lymphocytes, plasma cells, and histiocytes beneath the endocardium, and to a lesser extent in the myocardium in cases of hemolytic streptococcus infections. Similar lesions were found in the kidney, liver, lungs, spleen, and pancreas in their cases. They suggested that these lesions were due in part to an antigen-antibody reaction. Although the lesions which they demonstrated in the heart were focal, it seems possible that a diffuse interstitial myocarditis could develop on the same basis.

"Sulfa drug" sensitivity as a possible etiologic factor appears remote. There was a history of chemotherapy at 3 months of age, and as far as it is known there was no further history of the drug until the terminal illness. Since the infant died 15 hours after admission, it would seem unlikely that the amount of myocardial exudation observed could possibly have occurred in that brief time. It is also evident that the patient had cardiac involvement at the time of admission.

The rôle of the inoculation in case 3 cannot be evaluated except that it might possibly have been an etiologic factor.

Although case 4 was the only one in this series in which the patient lived long enough for an adequate clinical study, there is no lead to a possible etiologic factor.

The pulmonary lesions in all four cases were essentially similar. Histologically, they did not represent severe inflammatory processes, but rather a terminal interstitial pneumonia. It is also interesting to note that all blood cultures were negative, although pathogenic organisms were cultured from the lungs.

SUMMARY

In four instances of interstitial myocarditis in children, three were of the diffuse interstitial type and one could be included in the granulomatous group.

At present there is no known cause for myocarditis of this type.

The main purpose of this paper is to direct renewed interest toward myocarditis of an interesting and poorly understood type, which causes death in infancy as well as in later life.

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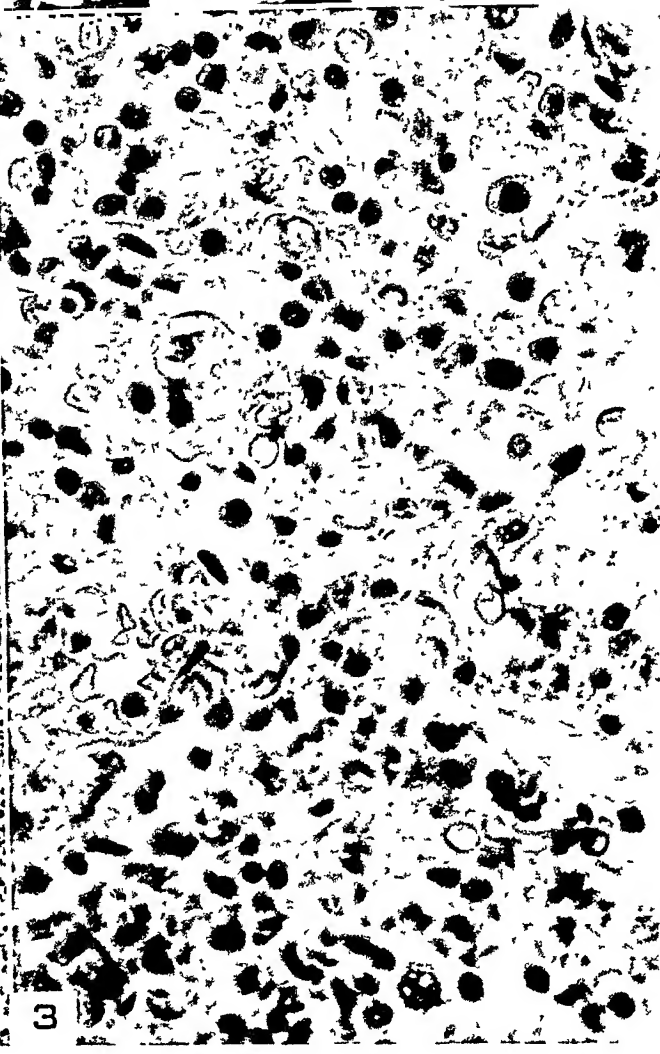
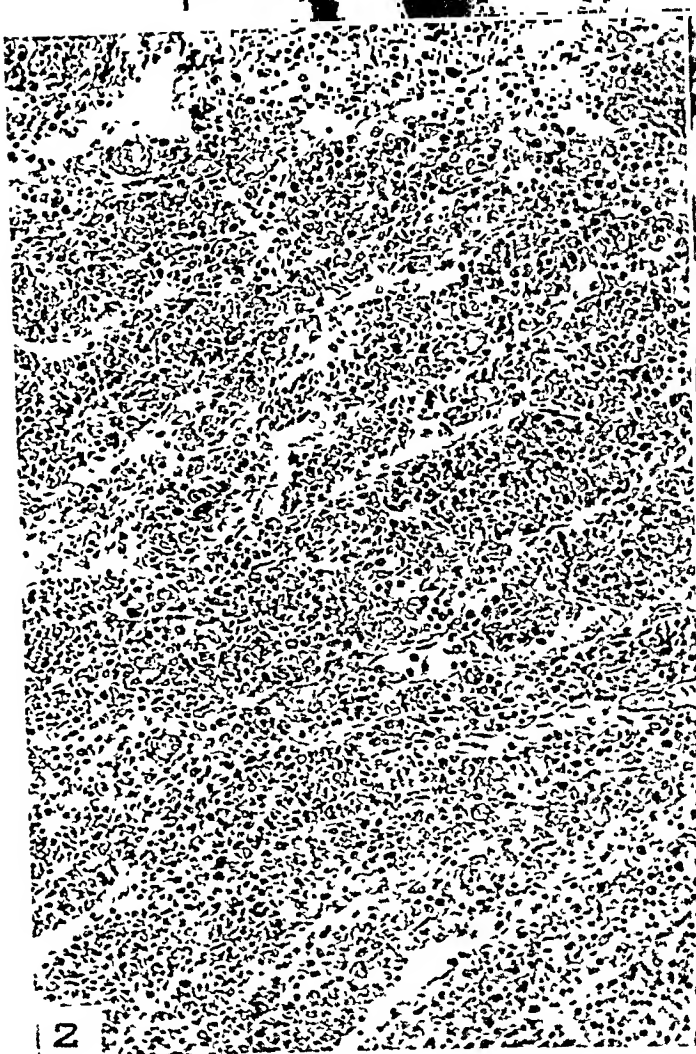
DESCRIPTION OF PLATES

PLATE 202

FIG. 1. Case 2. Photograph of organs *in situ*. Of note is the relative size of the heart.

FIG. 2. Case 1. Low-power view of the left ventricle in the area of greatest damage, showing leukocytic infiltration, abundant capillaries, and loss of myocardial fibers. Hematoxylin and eosin stain. $\times 140$.

FIG. 3. Case 1. Higher power view of the same area seen in Figure 2. Hematoxylin and eosin stain. $\times 500$.



Diffuse Interstitial Myocarditis

PLATE 203

FIG. 4. Case 2. A representative section of the left ventricle, showing the leukocytic infiltration. Hematoxylin and eosin stain. $\times 100$.

FIG. 5. Case 2. A higher power view of the same area seen in Figure 4, showing loss of myocardial fibers. Hematoxylin and eosin stain. $\times 500$.

FIG. 6. Case 3. Low-power view displaying a diffuse interstitial exudate. Hematoxylin and eosin stain. $\times 180$.

FIG. 7. Case 3. Low-power view of another area, showing a focal collection of lymphocytes. Hematoxylin and eosin stain. $\times 180$.

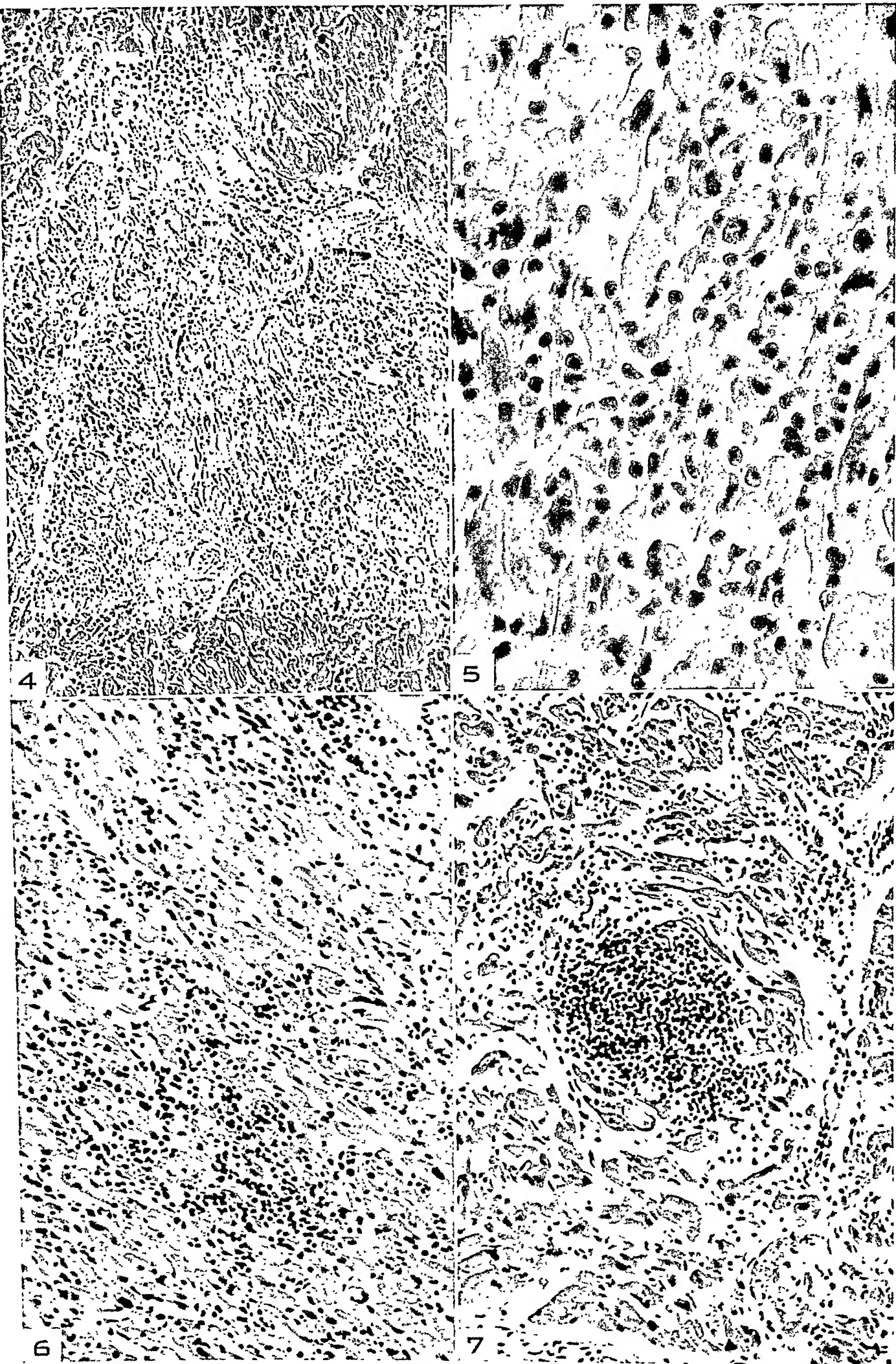


PLATE 204

FIG. 8. Case 3. Photograph of organs *in situ*. Of note is the relative size of the heart, similar to that of Figure 1.

FIG. 9. Case 3. Low-power view of an area of fibrosis with minimal exudation. Hematoxylin and eosin stain. $\times 250$.

FIG. 10. Case 3. A higher power view of the same area seen in Figure 9, showing types of exudative cells—lymphocytes, plasma cells, macrophages, and polymorphonuclear leukocytes. Hematoxylin and eosin stain. $\times 500$.

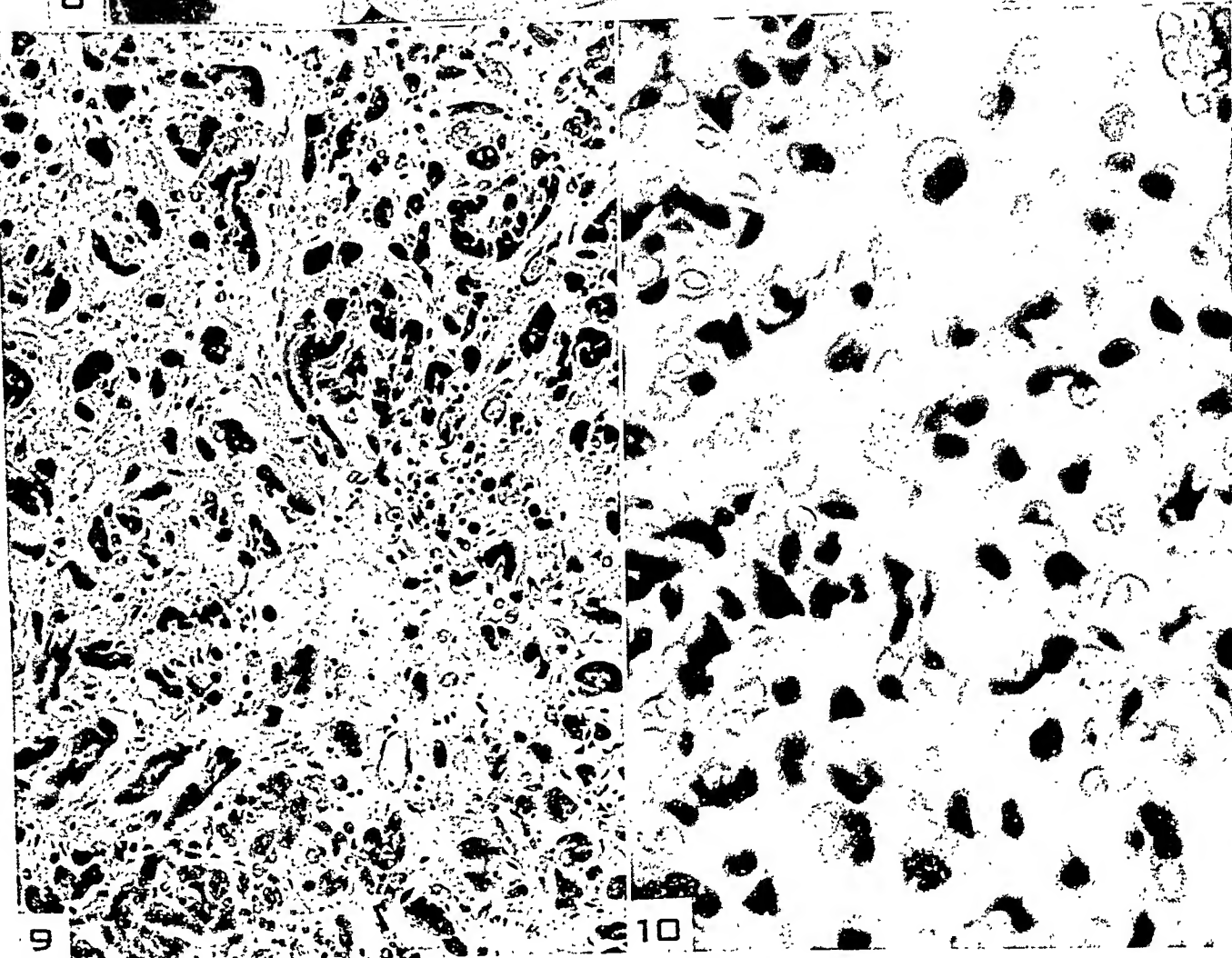


PLATE 205

FIG. 11. Case 4. Photograph of organs *in situ*. Similarity to Figures 1 and 8 may be noted.



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House

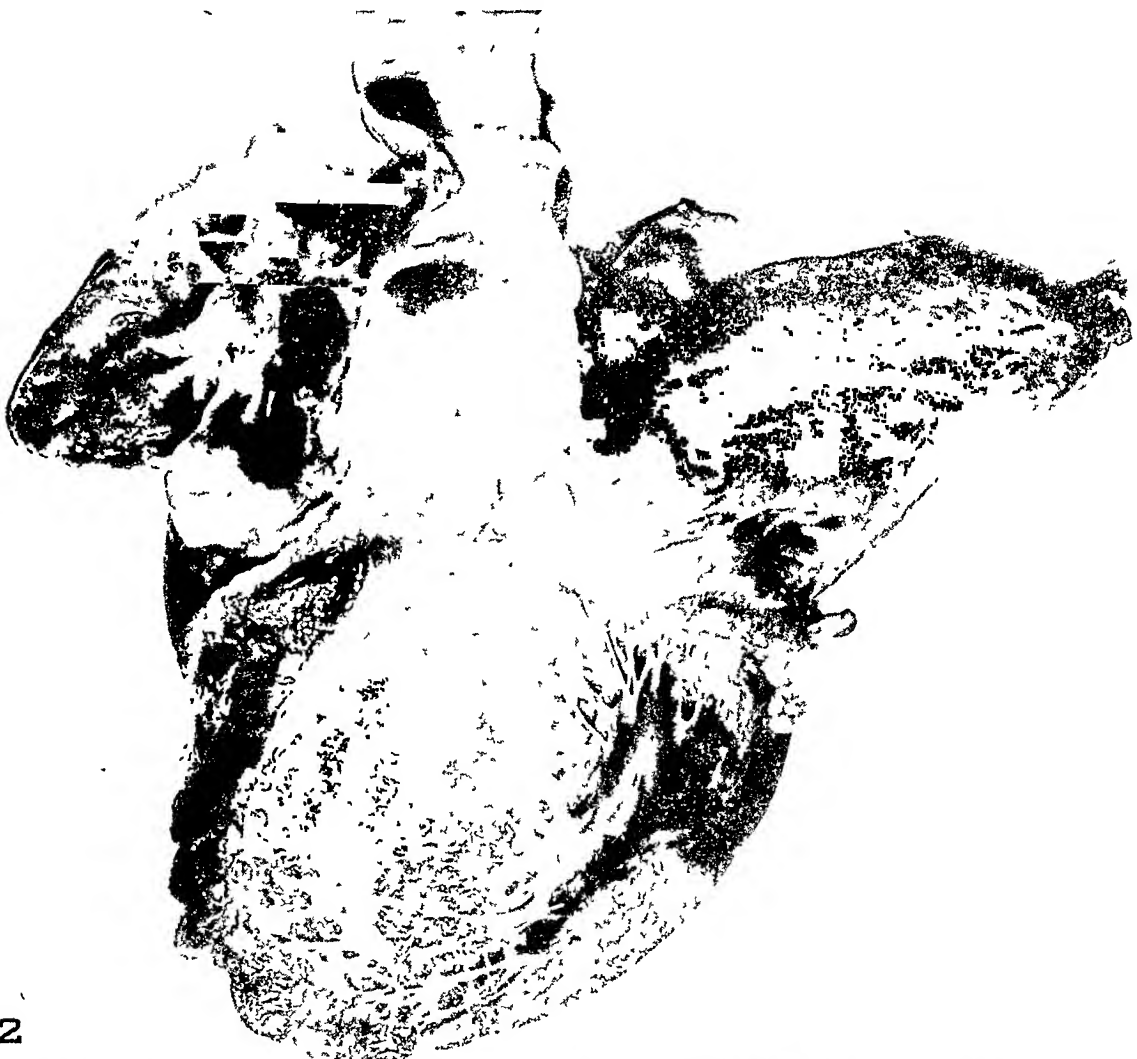
Diffuse Interstitial Myocarditis

PLATE 206

FIG. 12. Case 4. View of the open left ventricle. There is a generalized opacity of the endocardium (fibro-elastosis).

FIG. 13. Case 4. Low-power view showing the diffuse interstitial leukocytic infiltration. Hematoxylin and eosin stain. $\times 140$.

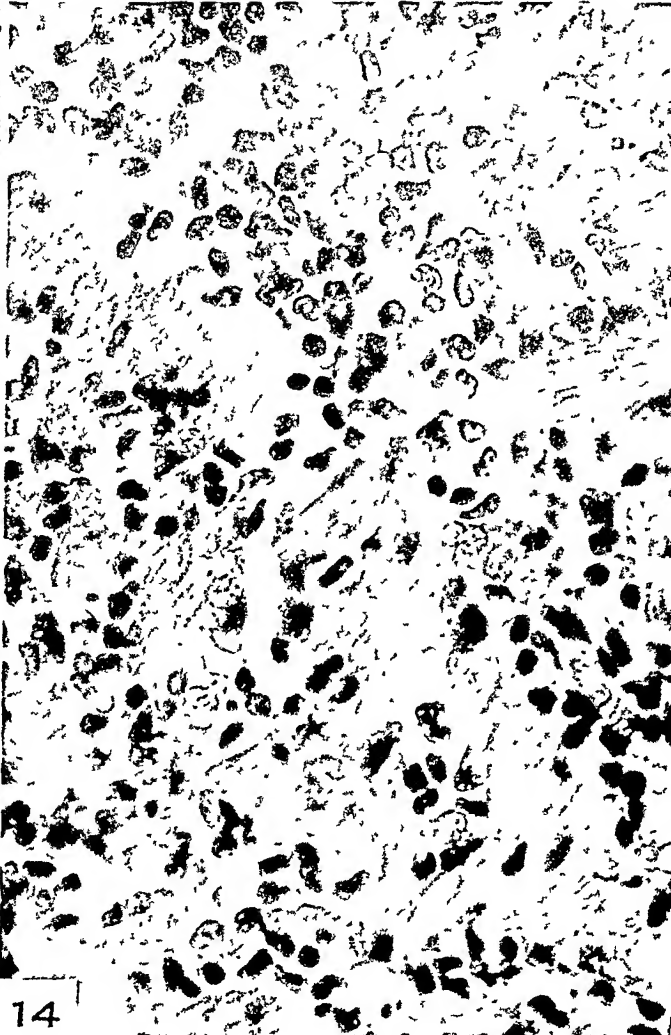
FIG. 14. Case 4. Higher power view of the same area seen in Figure 13. The exudate is predominantly lymphocytic in this field. Hematoxylin and eosin stain. $\times 500$.



12



13



14

STRUCTURE OF THE GLOMERULUS OF THE HUMAN KIDNEY *

J. F. A. McMANUS, M.D.

*(From the Department of Pathology, The Medical College of Alabama,
Birmingham 5, Ala.)*

Present opinion, following the studies of McGregor,¹ Bell,² and others, considers the glomerulus of the human kidney as a simple contortion of capillaries, lacking any stromal elements. In direct opposition to this, Zimmermann³ gave a description of the mesangium—a connective tissue framework for the glomerulus. Kimmelstiel and Wilson⁴ and Goormaghtigh⁵⁻⁷ utilized the concept of mesangium in discussions of glomerular alterations in disease. Difference of opinion on a matter so fundamental as glomerular structure suggested a review of the matter with the periodic acid-Schiff's reagent (PAS) technic.^{8,9}

This paper describes and illustrates the results of a study of the human glomerulus with particular reference to the basement membrane and the stromal elements or mesangium. The method of study is the coloring of paraffin sections by the PAS technic with which there is a selective coloring of the basement membrane of the renal tubule and glomerulus.^{8,9} The report includes the description of the glomerulus in kidney disease of several types.

MATERIALS AND METHODS

Much of the material came from the autopsy and surgical series of the Jefferson-Hillman Hospital. Slides from the files of the old Hillman Hospital (1921-1944 inclusive) were colored by PAS after removing the coverslips in xylene and decolorizing in 1 per cent hydrochloric acid in 70 per cent alcohol. Cases of traumatic uremia and some normal material had been collected during the European campaign of World War II. Other specimens were sent in by various pathologists.

The time or type of fixation is not crucial in the study of the glomerular basement membrane with the PAS technic. The basement membrane is preserved in tissues in which autolysis is advanced. Zenker's formol is the best of the routine fixatives for the glomerular basement membrane and Bouin's fluid and Holmgren's^{9a} 4 per cent sub-acetate of lead are useful because of their ability to preserve carbohydrate in sections. Dehydration by autotechnicon † preserves somewhat less of the PAS-colored material in the glomerular basement membrane than

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† The Technicon Co., 215 E. 149th St., New York 51, N.Y.

the slower technics. The staining technic for the basement membrane is that previously described in detail.⁹

Where blocks were available, sections were cut generally at 6 μ . Thin sections at 3 μ and thick sections at 25 μ were cut on occasion. A few serial sets were made.

THE NORMAL GLOMERULAR BASEMENT MEMBRANE

In thick sections, the glomerular loops were seen as a tangled skein of tubes of inconstant caliber. Unequal dilatation of the loops occurred after death from heart failure and the larger loops could be traced to the efferent vessel. All of the loops were covered with a deeply colored basement membrane which showed apparent points of concentration where loops lay in contact.

In 3 μ sections, it could be seen that the afferent vessel divided into a number of branches, each having a basement membrane continuous with that of the arteriole. If the loop was situated peripherally near the root of the tuft, its basement membrane connected with that of the parietal layer of Bowman's capsule. A short distance after the division into branches, *i.e.*, the formation of loops, or at times immediately, the loops became contorted and could not be followed adequately.

In sections 6 to 8 μ thick, a loop could be seen at times to curve about a central axis (Fig. 1). The basement membrane covered the loops and an intervening axial space. Studying this same area in the thinnest sections, it could be seen that there might be a nucleus in the central (axial) portion or, more commonly, the space might appear empty except, perhaps, for cytoplasm (Fig. 2). Frequently the axial cells projected knobs of their cytoplasm into two or more adjacent loops. In some cases the portion of the basement membrane which lay over the axial cells, *i.e.*, between the loops, could be seen to be thickened (Fig. 2), producing one variety of the "axial thickening" of Kimmelstiel.⁴

It was impossible to define the cells in the axial space as endothelium or as epithelium. Their nuclei stained like endothelial cell nuclei with phosphotungstic acid hematoxylin. Calling them "third type" cells might serve for purposes of description while prescinding from the question of their origin. No myofibrils were seen in their cytoplasm, either in material taken for biopsy or from autopsies performed from 30 to 45 minutes after death, after any fixative, or with any stain.

Sometimes, as McGregor¹ pointed out, a coarse axial fiber in the glomerulus could be traced to the parietal basement membrane of Bowman's capsule, this being especially noticeable when the latter was thickened (Fig. 3). That represented the only connection that could

be recognized between the axial space and an extraglomerular structure.

In summary, the glomerular basement membrane in the normal human kidney is formed from the basement membrane of Bowman's capsule which becomes attached to the arterioles of the glomerular root. Covering the tuft, the glomerular basement membrane encloses: (1) the capillaries of the loops which have no basement membrane of their own; (2) the endothelial cells of the capillaries; (3) the axial space between the loops where the basement membrane is reflected over the loops; (4) the infrequent axial or third type cell in these spaces; and (5) occasional fibers which pass into the glomerulus from Bowman's capsule.

It is possible that only epithelial basement membrane is being shown in the glomerulus with PAS. Capillary linings are shown elsewhere. The studies of abnormal glomeruli to be described make this improbable.

THE GLOMERULUS IN SOME DISEASES

The glomeruli in renal diseases of specific types have been studied because of the apparent involvement of the axial space or of the description of mesangial lesions. The series includes:

Diabetes mellitus	4 cases
Intercapillary glomerulosclerosis	6 cases
	(2 nondiabetic)
Acute glomerulonephritis	10 cases
Eclampsia	16 cases
Pregnancy controls	12 cases
Wounding (crush) kidneys	10 cases*

Thin microscopic sections of kidneys in diabetes mellitus, colored with PAS, were the best material in which to recognize the axial space. The axial area always was prominent and colored strongly with PAS. It usually appeared finely fibrillar, as Bell² described, although Bell considered the fibrils to be intracapillary. These fibrils were colored by PAS while the fibrils of muscle cells were not. The intercapillary glomerulosclerosis of Kimmelstiel and Wilson,⁴ when it occurs in diabetes, appears to be an accumulation of a PAS-positive material in the axial space and need not be accompanied by thickening of the basement membrane of the glomerulus.

The kidneys with intercapillary glomerulosclerosis showed, in the

* These 10 cases were part of a group of 35 battle casualties collected in a study of the kidney after severe wounds. The 10 were selected for review in this article because of early autopsy and adequate fixation.

earliest stages, the deposit of a strongly colored hyaline material in the axial space. The later stages showed gradual increase of the intercapillary hyaline material and usually all or many stages could be seen in one kidney. Finally, the loops were situated peripherally about the hyaline mass. The largest or most involved loops had an inner limit or "basement membrane" made up of the hyaline mass. The peripheral true basement membrane might not be thickened or distorted (Fig. 4), although this happened frequently in the non-diabetic cases. Sometimes the hyaline mass included nuclei as though this were some storage process associated with an increase of cells of the third type.

The cases of acute glomerulonephritis showed the customary increased glomerular cellularity. A proportion of this appeared to be due to endothelial proliferation. A striking feature of the situation of some of the additional cells, including polymorphonuclear leukocytes, was their localization in the axial space of the glomerulus (Fig. 5). Pyknosis of nuclei might be seen, possibly those of third type cells.

The typical glomerulus in eclampsia in thin sections appeared lobulated, the individual capillary loops being separate and discrete. This lobulation was in contrast to the lobation of acute glomerulonephritis in which the glomerulus appeared divided into two, three, or four main portions or lobes.

The capillary loops were very prominent and were covered with numerous epithelial cells. I am not sure that there had been an actual increase in the epithelial cells. It may be that autolysis had not occurred and that the normal number of cells remained. The loops themselves had the appearance of being turgid or erectile. There was a constant vacuolation of the axial space and this sometimes involved the endothelial cells (Figs. 6 and 7). There was no thickening of the basement membrane or proliferation of the endothelial cells, and there was no cellular infiltration of the mesangium. The control pregnancy material showed no similar reticulation.

The vacuolation of the axial space which produced reticulation of the glomerulus in eclampsia was a striking and characteristic lesion. It was not due to fatty change or infiltration in 2 cases available for frozen sectioning. Two cases which were clinical lipoid nephrosis—actually early chronic glomerulonephritis—showed reticulation of the axial space besides adhesions and thickening of the basement membrane. In these cases reticulation was due to triglyceride fat, colorable with sudan IV, as Bell¹⁰ mentioned in his study of lipoid nephrosis.

I have found an occasional glomerulus in some cases of hyperten-

sion which showed reticulation, not due to lipoid. The lesion in these cases is restricted to a few glomeruli and is not a diffuse finding as in eclampsia.

DISCUSSION

The arrangement of the basement membrane in the normal glomerulus, as demonstrated by PAS, corresponds more closely to the description of Zimmermann³ than to that of McGregor.¹ The latter recognized the thickened portions of the glomerular basement membrane and the localization of a third type cell at these points, but considered these thickenings as "orifices into" or "passageways between" loops. The possibility of the third type cell being a "connective tissue cell" was mentioned by McGregor. Zimmermann identified the third cell as a fibrocyte, presumably a connective tissue cell.

The glomerulus is best visualized as a set of discrete, non-anastomosing, vascular channels invaginating the basement membrane of the Bowman's capsule. Rare pericapillary cells exist in the intercapillary or axial space, where the basement membrane is reflected over adjacent loops. No evidence of a double basement membrane is found, except in injected kidneys. The lesions for which MacCallum,¹¹ Goormaghtigh,⁵⁻⁷ and others supposed a double basement membrane can be explained by the arrangement described.

Goormaghtigh considered the third cell as a muscle cell controlling the "tonus" of the glomerulus.⁵⁻⁷ He described multiplication of the myocytes of the mesangium in acute glomerulonephritis and hypertrophy and hyperplasia of these cells in eclampsia. The changes in both these conditions were said to be similar to the changes in the kidney in "traumatic uremia" or the "crush" kidney (Fig. 1). I have not found the changes described by Goormaghtigh. The glomerulus in traumatic uremia is little if at all involved and shows no resemblance to the glomeruli in either acute glomerulonephritis or eclampsia.

Acute glomerulonephritis is an acute inflammation of the glomerulus which involves the capillaries and the axial space. The localization of inflammatory cells in this space and subsequent fibrosis would explain the "intercapillary" lesions (Fig. 8) of MacCallum.¹¹ The proliferating endothelial cells within the capillaries in acute glomerulonephritis become outlined with PAS-positive material. This "arteriole-like" appearance of the loop may furnish the basis for later intracapillary fibrosis (Fig. 9). Both intercapillary and intracapillary lesions may be seen in one glomerulus (Fig. 10).

The specific lesion of the glomerulus in eclampsia is restricted to the cells of the mesangium or axial space. These cells become swollen

and vacuolated. The resulting "reticulation" of the glomerulus in eclampsia was described¹² and illustrated¹³ by Dunn. Jaffe¹⁴ called the glomerular lesion in eclampsia an "edema of the tuft," which would agree with the present description.

Intercapillary glomerulosclerosis could be the result of "trapping" a glycoprotein in the axial space. Practically identical findings were seen in 2 cases of chronic glomerulonephritis (probably type II of Ellis¹⁵) and one wonders whether a chemical or serologic similarity could be found between the urinary protein in the diabetic and glomerulonephritic cases which show similar glomerular lesions. The fat in the axial space in lipoid nephrosis might be accumulated in a similar fashion or might be the result of an injury to the mesangial cells.

Allen¹⁶ considered and dismissed the possibility of a mesangial origin for the hyaline material in intercapillary glomerulosclerosis. Reticulin stains show a lamination of the hyaline deposit in the glomerulus which Allen interpreted as evidence that the material is formed by duplication of the internal capillary wall. I do not think this is the mode of development of the lesion. In the first instance, the internal or axial capillary wall does not color with PAS (Fig. 2). On occasion, the hyalin of the intercapillary lesion can be seen to inject, as it were, one leaf of the axial space (Fig. 11). Furthermore, I do not see how it is possible in sections to differentiate fibrils from irregularly deposited hyaline material, laid down in strata.

The questions of nature and function of basement membrane are raised in pointed fashion by these studies on the glomerulus. In its original morphologic use, the term refers to hyaline linear structures visible microscopically at epithelial-connective-tissue and epithelial-capillary junctions. Empirical methods of staining have produced no definite evidence for the generally held belief that basement membrane is related to intercellular substance, known to contain the carbohydrates hyaluronic acid and chondroitin-sulfuric acid.

The glomerular basement membrane fits the criterion of position at an epithelial-capillary junction. The strong coloring which it shows with Schiff's reagent after periodic acid suggests a carbohydrate constituent.

SUMMARY AND CONCLUSIONS

The basement membrane in the normal glomerulus as shown with the PAS technic derives from Bowman's capsule and attaches to the arterioles at the glomerular root. It encloses capillary loops and certain infrequent stroma cells of the mesangium, the latter in the intercapillary or axial space.

The axial space is prominent in diabetes mellitus. It appears finely fibrillar, as was described by Bell.

Marked involvement of the axial space can be seen in various disease conditions. These can be tabulated as follows: Acute inflammation in acute glomerulonephritis; glycoprotein accumulation in intercapillary glomerulosclerosis; vacuolation and reticulation in eclampsia; lipoid accumulation in "lipoid nephrosis."

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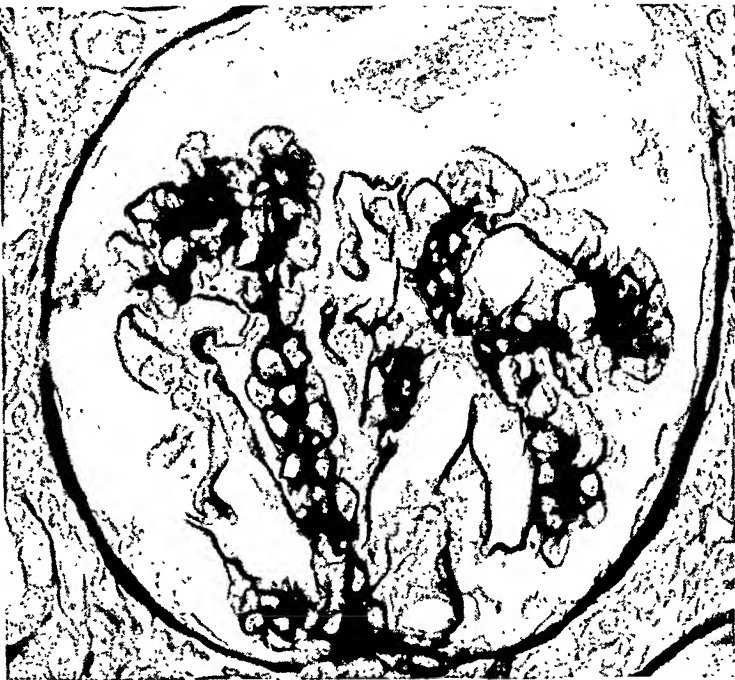
[Illustrations follow]

DESCRIPTION OF PLATES

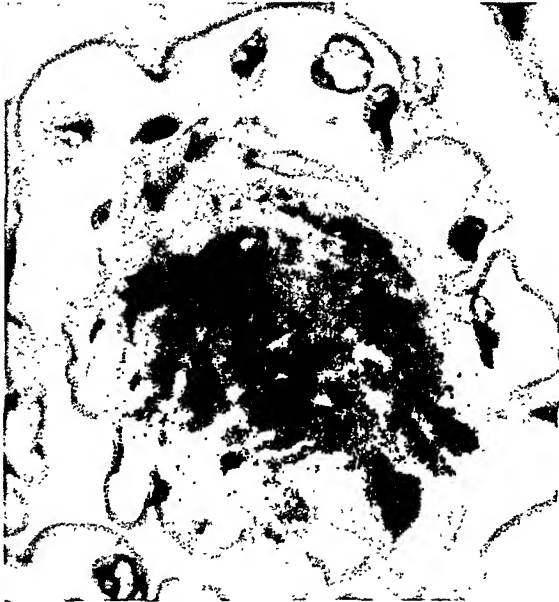
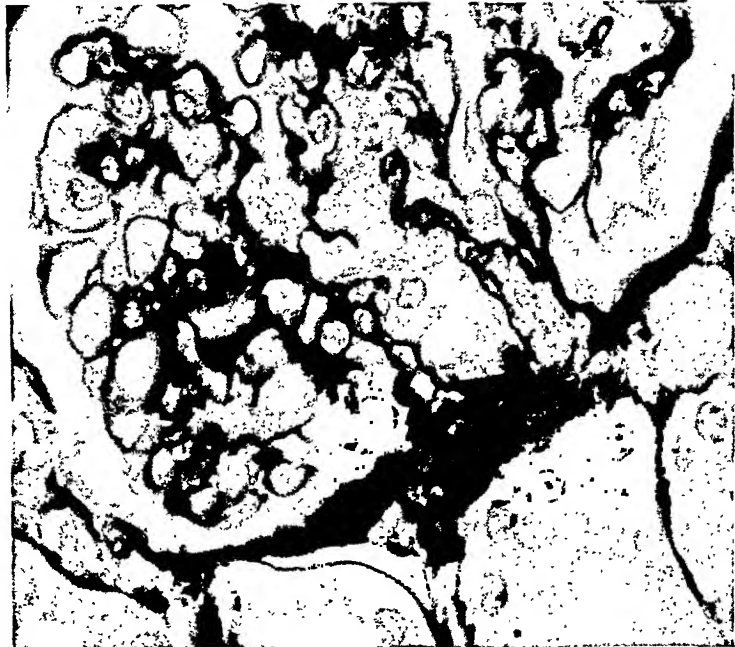
PLATE 207

- FIG. 1. Glomerulus from "crush" kidney after severe wounding. Loops at left center are arranged about a central axis. Periodic acid-Schiff's reagent (PAS). \times about 300.
- FIG. 2. Glomerular detail. The patient was a healthy adult male in shock from a chest wound 18 hours before death. Axial space is left as a T-shape by reflection of basement membrane over adjacent loops. PAS. \times about 800.
- FIG. 3. Same case, same glomerulus and technic as shown in Figure 2. Coarse fiber enters from the parietal basement membrane on the right. PAS. \times about 300.
- FIG. 4. A-657. Intercapillary glomerulosclerosis in arteriolonephrosclerosis. Centrally placed, hyaline, lamellar material with intact peripheral basement membrane. PAS, hematoxylin counterstain. \times about 800.
- FIG. 5. Acute glomerulonephritis. Proliferation of endothelial cells. Infiltration of axial space. PAS, hematoxylin. \times about 800.
- FIG. 6. Eclampsia. Vacuolation of axial space extending to endothelium. PAS, hematoxylin. \times about 800.

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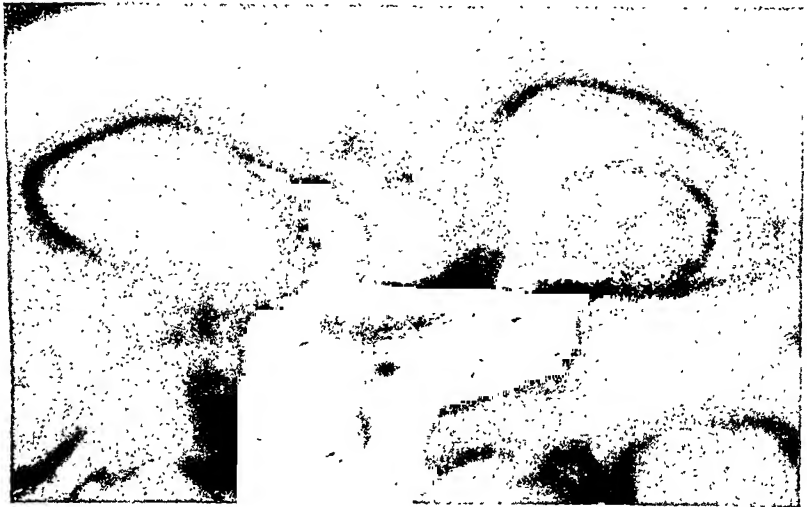
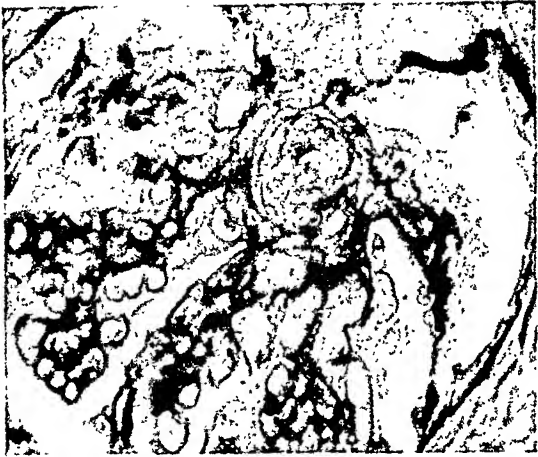


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PLATE 208

- FIG. 7. Eclampsia. Separate capillary loops with fine reticulation of axial space. PAS, hematoxylin. \times about 300.
- FIG. 8. Subacute glomerulonephritis. Early fibrosis of axial space with intact peripheral basement membrane in capillary loops. PAS. \times about 250.
- FIG. 9. Same case as shown in Figure 8. Diffuse intracapillary and axial fibrosis in progress. PAS. \times about 250.
- FIG. 10. Subacute glomerulonephritis. On the right of the glomerulus the peripheral loops are spared, producing intercapillary disease. To the left, the loops are involved diffusely, producing intracapillary disease. PAS. \times about 250.
- FIG. 11. Intercapillary glomerulosclerosis in a hypertensive diabetic patient. Of note is the peripheral displacement of loops with little change in caliber. PAS. \times about 250.
- FIG. 12. Higher magnification of Figure 2. \times about 1250.



McManus

Structure of the Glomerulus



CELLULAR CHANGES IN RABBITS DURING ANTIBODY FORMATION

I. RESPONSE TO *EBERTHELIA TYPHOSA* *

CRICHTON McNEIL, M.D.

*(From the Department of Pathology, University of Utah School of Medicine,
Salt Lake City, Utah)*

Pathologists are well aware that certain cell groups, constituting a granuloma, represent a reaction of tissues to antigen of a specific type. In most instances this tissue reaction is successful in retarding the growth of living agents or of eliminating the antigen, and this we attribute to an immune response of cells. However, while we are keenly aware of cell reactions representing an immune response, we are almost entirely unaware of cell reactions preceding immunity when antibodies are developing.

In the past 4 decades many attempts have been made to locate the site of antibody formation so that the pathologist might identify clearly the early stages of a disease process, but at present confusion still exists. Today a survey shows that there are four theories. One contends that cells of the reticulo-endothelial system phagocytize the antigen and produce the antibody. This theory is supported by "blockade" experiments of the reticulo-endothelial system,¹⁻⁵ by dye-tagged proteins,⁶ and by the removal of organs.¹⁰⁻¹² Another theory supports the lymphatic cell as the locus of antibody formation on the basis of serologic studies showing slightly more agglutinin in lymph nodes than in serum.¹³⁻¹⁶ A third opinion recognizes that aggregates of plasma cells contain much more antibody than other tissues.^{2,17} A fourth investigator has submitted evidence that cells are not necessary for antibody formation.²²

Therefore, under conditions of inflammation, the pathologist is not sure what interpretations are suitable when he observes unusual concentrations of reticulo-endothelial cells, lymphocytes, or plasma cells. It is toward a solution of this problem that the present report is directed.

node were selected for study as being ideal because of convenience and consistency of lymph node size.

As a control study, to obtain baseline figures, right and left deep cervical lymph nodes of 5 normal rabbits were removed and divided. One half was immediately fixed for a paraffin section and the cut surface of the other half was carefully scraped with a sharp knife so that the scrapings floated into normal rabbit serum. In this way the entire contents of one-half of the node could be suspended in the serum for the preparation of smears which were quickly dried and stained by Wright's method, using buffered water of pH 6.9. Evenly distributed cells were always obtained and upon these preparations differential cell counts could be performed easily and rapidly. This method gave permanent mounts which could be restudied at any time, and for this study seemed more satisfactory than the supravital technics.^{23,24} One thousand cells were counted on each side, thus giving the observer a proper survey of cell types. Slight changes in numbers of cell types could readily be recognized. Skin sections, lymph node sections, and peripheral blood smears were obtained also.

Figure 1 illustrates the six cell types which may be seen in the lymph node smears. Table I shows the distribution of these cells per 1000 and includes disintegrated cell elements. Heterophil granulocytes are very scarce in the normal lymph node smears.

It seemed advisable to study peripheral blood smears for possible reflected changes in cell types. Table II shows the results of differential and total blood counts of 11 normal rabbits. These counts correspond quite well with the figures published by those who have done hundreds of counts.^{25,26} Emphasis was placed upon the differentiation of large and small lymphocytes so that any alteration might be correlated with lymph node smears.

CHANGES IN SKIN, LYMPH NODES, AND BLOOD OF RABBITS INJECTED WITH ANTIGEN (*EBERTHELLA TYPHOSA*)

Rabbits were injected intradermally over the right mandible with 0.5 cc. of killed *Eberthella typhosa* bacilli in concentrations given below. A single dose was given to all at the same time and then the animals were killed at intervals as described below. The following materials were saved for study: (1) skin for sections stained with hematoxylin and eosin, (2) lymph nodes (right and left) for hematoxylin and eosin preparations, smears, and extraction for antibody titer, (3) serum for antibody titer, and (4) blood smears for differential counts.

Experiment 1. Five rabbits received 0.5 cc. of the vaccine containing 2 : 1 billion bacilli per cc., as superficially as possible into the skin

over the angle of the right mandible. A sixth animal, as a control, received a sterile saline injection in the same location. The 5 antigen-injected rabbits were bled, blood counts were taken, and the animals were killed by air injection on the 2nd, 5th, 8th, 10th, and 15th days. During the course of the experiment none of the animals showed untoward effects of the injection and the control rabbit was sacrificed and examined on the 3rd day. All animals were autopsied immediately

TABLE I
Differential Lymph Node Cell Counts (1000 Cells) on 5 Normal Rabbits

Rabbit no., site	Small lymphocytes	Large lymphocytes	Basophilic lymphocytes	Lymphoblasts	Pseudo-eosinophils	Reticulum cells	Defective platelet cells
1. Right	736	207	48	9	0	0	0
1. Left	793	18	2	0	1	0	177*
2. Right	890	56	30	20	0	4	0
2. Left	909	55	22	12	1	0	1
3. Right	801	165	21	9	1	0	3
3. Left	770	200	15	12	2	0	1
4. Right	816	163	10	0	0	0	2
4. Left	785	157	11	14	0	0	53
5. Right	904	64	4	12	0	1	15
5. Left	859	97	14	7	1	1	22
Average	826	118	17.7	11.3	0.6	0.6	25.4

*Defective smear.

the lymph nodes and to correlate these changes with cellular changes. In the first three groups of animals serologic tests were carried out using the usual tube dilution technic. Maximum agglutination occurred using 5 billion bacilli per cc. In experiment 4, Heidelberger and Kabat's²⁷ agglutination and agglutinin nitrogen method was used.

RESULTS

Skin

A uniform progressive change occurred in the skin of all of the antigen-injected animals, and this response may be described in the following phases extending from 6 hours to 15 days: *Phase 1*, a diffuse

TABLE II
Total and Differential (per 1000 cells) Peripheral White Blood Cell Counts on Normal Rabbits

Rabbit no.	Total white blood cells	Small lymphocytes	Large lymphocytes	Pseudo-eosinophils	Neutrophils	Mono-cytes	Baso-phils	Eosino-phils
1	8,650	13	26	46	1	3	8	3
2	5,900	27	20	49	1	1	2	0
3	7,200	26	28	42	0	2	2	0
4	8,000	40	16	40	0	2	2	0
5	9,400	32	23	41	0	0	4	0
6	4,500	44	9	35	2	3	6	1
7	9,800	32	23	45	0	0	0	0
8	12,800	40	12	45	0	0	2	1
9	8,750	23	17	57	0	0	2	1
10	5,500	18	17	60	0	2	2	1
11	7,600	31	20	41	0	4	3	1
Average	8,009	29.6	19.2	45.6	0.36	1.5	3.0	0.73

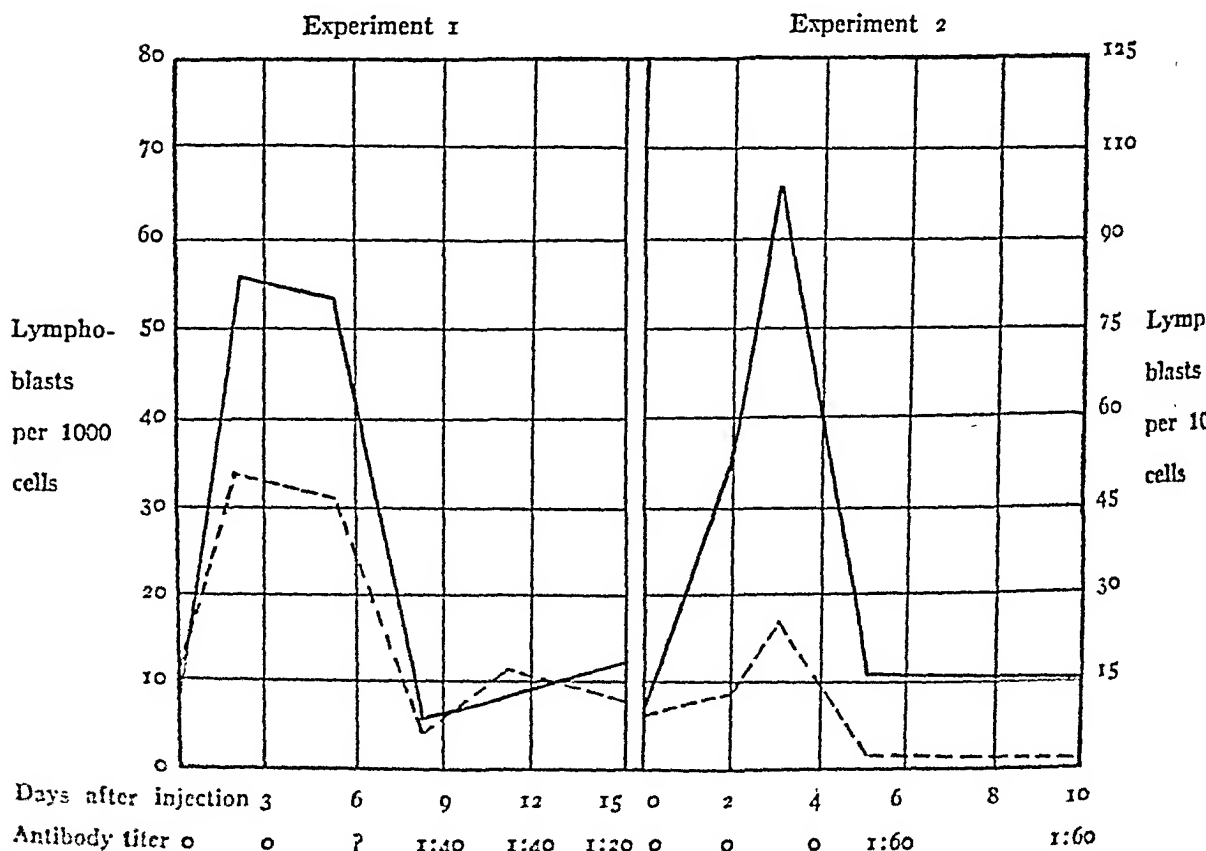
edema and infiltration of the upper corium with pseudo-eosinophilic and eosinophilic granular leukocytes and no trace of the injected bacilli in bacteria-stained sections. The duration of this change was 48 hours (Figs. 2 and 3). *Phase 2*, heavy concentrations of these leukocytes and focal collections in the deeper portions of the corium and in the superficial muscle layer. Infrequent large macrophages were present. The duration of this change was 3 to 6 days. *Phase 3*, return of the skin to normal except for one or two pinhead-sized yellow areas in the corium which on microscopic examination had a tiny, central, necrotic zone and a granulomatous capsule of macrophages and fibroblasts. Healing had all but been completed. The duration was 8 to 15 days.

Lymph Node Smears

Certain distinct changes in numbers and proportions of cells occurred in the lymph nodes during the course of the experiments. Table III presents the results of differential counts done on the right cer-

and decrease rather rapidly until at 48 hours their number is reduced to normal.

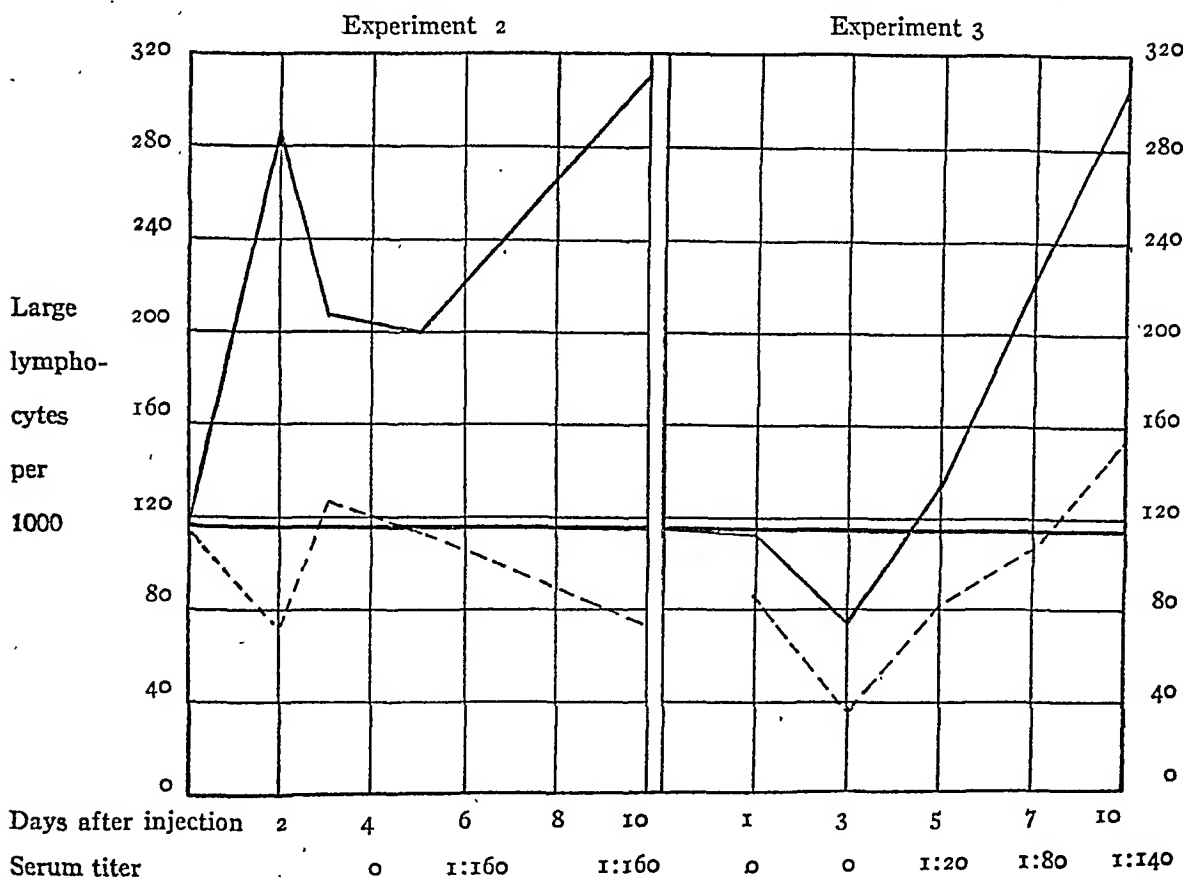
At the time that the eosinophilic cells decrease (48 hours), the lymphoblasts go through an interval of hyperplasia which lasts for 3 days or more, during which there is a five-fold increase in their number. Text-Figure 1, which compares the numerical changes in lympho-



Text-Figure 1. Lymph node differential counts. The curves show the sudden rise and fall of lymphoblasts between the second and sixth days after injection. The solid line indicates results on smears from the right nodes, and broken line, from the left nodes.

blasts with the time interval in experiments 1 and 2, shows this diagrammatically. The diagram includes the differential counts done on the left cervical nodes and shows that a slight simultaneous hyperplasia of lymphoblasts is present in these nodes too. Table III shows that when the experiment is repeated several times the number of lymphoblasts decreases gradually over 4 to 6 days, while Text-Figure 1 shows a precipitous drop in lymphoblasts in each experiment. It must be kept in mind that these experiments include only a 15-day interval, which might be referred to as the primary phase of cellular changes in the rabbit.

Basophilic lymphocytes, which have been compared to the plasma cell, share in the hyperplasia with the lymphoblasts but at a slightly later interval. During the first two experiments it was thought that this basophilic cell increase was an isolated observation, but the aggregate of experiments shows a small but definite band of hyperplasia from the 5th to the 7th day just at the time of appearance of an agglu-



Text-Figure 2. Lymph node differential counts. The solid line indicates the increased number of large lymphocytes in smears from the right nodes after lymphoblastic hyperplasia. The broken line shows the number of large lymphocytes in smears from the left nodes.

tinin titer. This is not to be interpreted as a definite correlation at this time.

The evaluation of the changes in number of the large lymphocytes is somewhat more difficult but it may be stated generally that there appears to be a general increase after the interval of lymphoblastic activity. In order to bring this out it is necessary to examine Text-Figure 2, which shows the occurrence of these cells in experiments 2 and 3. Especially in experiment 3 the right lymph node smears show a steady rise in the number of large lymphocytes. The heavy black line across the center of the figure indicates the normal number of large lymphocytes per 1000 cells. It is observed also that it is these cells

which pinch off small globules of blue-staining cytoplasmic material, which has been referred to as "shedding."²⁸ It seems to me that wherever there are large lymphocytes this shedding phenomenon occurs, no matter what interval of antigen stimulation is represented. More shedding, of course, occurs when the number of large lymphocytes increases.

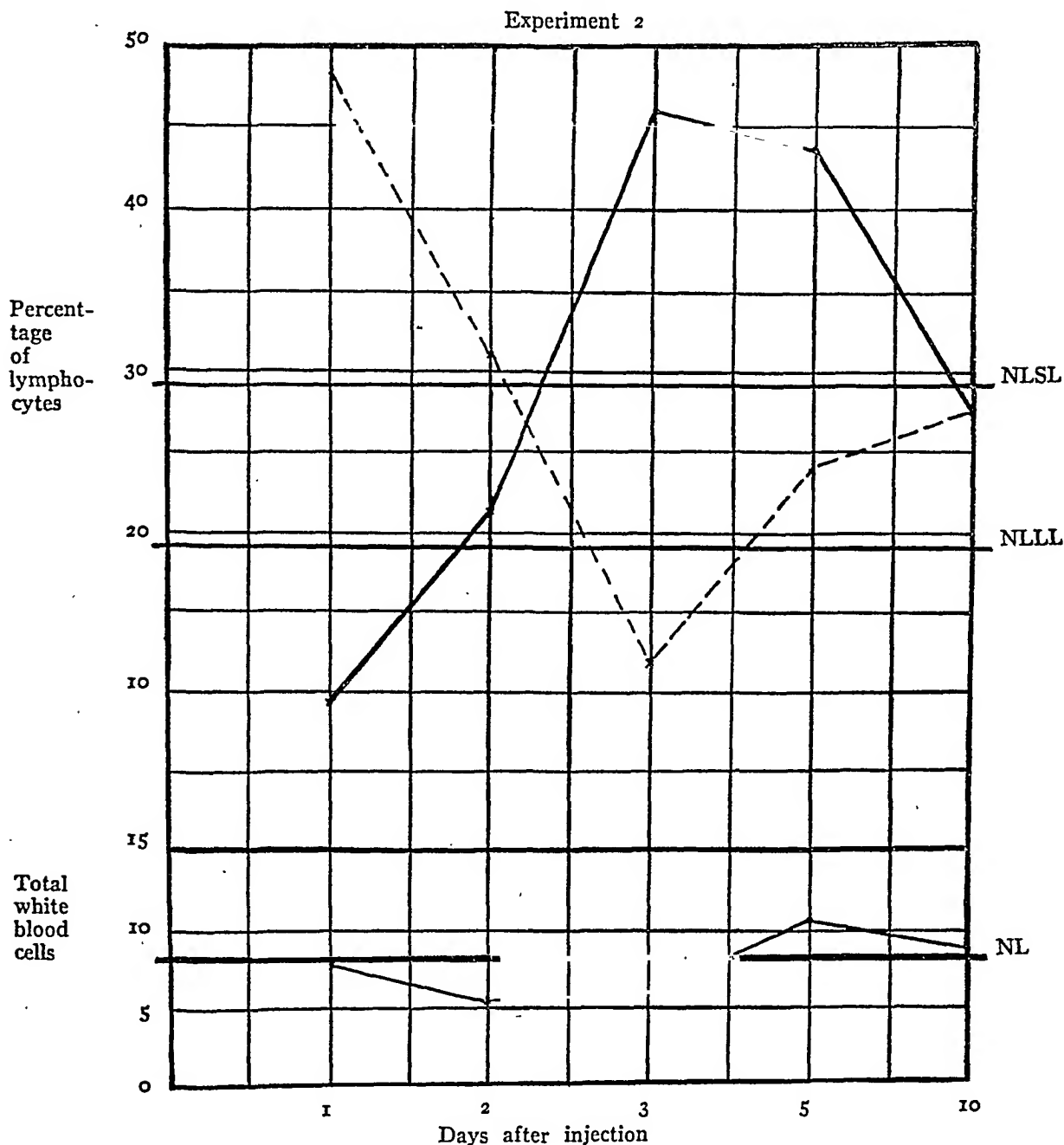
Another observation must be included for whatever interpretation might be deduced. Frequently, large lymphocytes were found to contain eosinophilic granules in their cytoplasm, not unlike those of eosinophilic leukocytes. This observation was made only at the stage when the eosinophils were disappearing (48 hours), and many granules were found loose in the smear.

Other cells differentiated in the nodal smears did not go through significant changes. Small lymphocytes are always present in large numbers and, of course, their number decreases as other cells become hyperplastic. But this does not measure the true status of this group since a fixed number of cells is counted. Comment on this point will be included in the section on the fixed lymph node material. Reticulum cells failed to show hyperplasia during the time interval represented. One would think that had this cell been stimulated to perform some function, a slight or moderate change in number would have occurred. Disintegrated cells seemed to increase somewhat during the longer intervals. Since these cells seemed to represent broken-down large lymphocytes with only the nuclear portion remaining, this increase is understandable because of the increase in large lymphocytes in the more prolonged experiments.

Lymph Node Sections

Cellular changes in the sections of lymph nodes were inconspicuous when compared to the changes in the smears. Edema and hyperemia of the hilar region and medullary zone (Figs. 4 and 5) occurred early in the right cervical nodes but not in the left. This was followed and accompanied by an infiltration of pseudo-eosinophilic leukocytes in the same areas. These leukocytes seemed to gather in groups of about 6 or 8 cells and were rarely found in the cortical sinuses. Some appeared to be undergoing disintegration, as was noted in the smears, but in the sections a few large cells with distinctly smooth, eosinophilic cytoplasm were usually nearby (Fig. 5). As seen under the oil immersion lens, they could have been either reticulum cells or large lymphocytes. One day later, at 72 hours, lymphoblastic activity was evident, scattered throughout the medullary zone and not, as might be expected, in the

primary follicles. On close inspection one could not make out any significant changes in the primary follicles despite the rather obvious activity going on in the central portion of the lymph node. There was a possible increase in small lymphocytes in the sinusoids. By the 5th day the lymph node sections appeared normal and the appearance was then identical to that of the left cervical nodes.



Text-Figure 3. Changes in percentage of large and small lymphocytes in the peripheral blood in antigen-injected rabbits. The upper portion shows the reversal of small lymphocytes and large lymphocytes after lymphoblastic hyperplasia. The lower portion shows essentially no change in total white count. NLSL, normal line of small lymphocytes. NLLL, normal line of large lymphocytes. Broken line, small lymphocytes; solid line, large lymphocytes.

Peripheral Blood Smears

At the time each animal was autopsied a total white and differential blood count was performed. Text-Figure 3 shows the significant changes, which appeared to be limited to the lymphocytic series for the most part. At the lower portion of this figure is shown the total white cell count, which did not deviate significantly from the normal. Above is shown the reciprocal behavior of the large and small lymphocytes during the course of the experiment. The change in preponderance from small to large lymphocytes is seen to occur at the interval of the increase of large lymphocytes in the lymph node smears. This seems to show then that these large lymphocytes in the nodes get into the general circulation rather quickly. A slight relative lymphocytosis occurred during the period of the reversal of small and large lymphocytes. Very little change in the number of cells of the granular series could be observed despite the rather intense leukocytic infiltration in the skin at the site of the injection of the antigen. The nature of the eosinophilic leukocytic change is to be studied in more detail in other experiments.

Scrologic Studies

Two sources of material, serum and ground-up lymph node in saline solution, were utilized in performing agglutination tests. The lymph node suspension was made by grinding a weighed portion of cervical node in 3 cc. of physiologic saline solution and sand, decanting off the cloudy fluid, and finally freezing and thawing the mixture three times. The sediment was then smeared to be sure that no intact cells remained, after which the material was centrifuged for 2 minutes at 1500 r.p.m. and the clear fluid separated for the tests. By this technic the number of mg. of lymph node material per cc. of physiologic saline solution can be determined readily. Table IV gives the agglutination

results by the usual tube dilution method applied to the serum and lymph node suspensions in experiment 3.

Calculation of the nitrogen content of the serum and the nodal suspensions showed that the serum contained fifteen times as much protein, but in spite of this correction the serum far outstripped the nodal material in its ability to agglutinate the antigen. Heidelberger and Kabat's²⁷ method was attempted in experiment 4, but the results will not be given because I could not be sure of their accuracy without the use of a constant temperature cold centrifuge. It is not the purpose of this report to contest the serologic aspects of serum versus lymph node antibody content, but the data collected so far do not agree with previous reports.^{13,18} It is believed that, given the proper equipment, the Heidelberger-Kabat method is probably more accurate than tube dilution methods.

SUMMARY

A method has been developed for performing differential cell counts on lymph nodes of rabbits. Differential counts of lymph nodes of normal rabbits were first determined, which gave baseline figures.

The following observations were made upon the skin and lymph nodes of rabbits injected cutaneously with *Eberthella typhosa* as antigen:

1. The bacteria disappeared from the corium within 6 hours and a diffuse pseudo-eosinophilic and eosinophilic infiltration took place which lasted for 48 hours.
2. During this same interval eosinophilic granulocytes appeared in the regional lymph node in large numbers and seemed to disintegrate there.
3. In the interval of 2 to 5 days a small focal granuloma formed in the skin at the site of injection and lymphoblasts became distinctly hyperplastic in the lymph nodes.
4. During the 5 to 7 day period basophilic lymphocytes increased moderately in number in the lymph nodes and the serum antibody titer started to rise.
5. From this time until the end of the experiments at 15 days, large lymphocytes usually were increased in number both in the nodal smears and in the peripheral blood smears. At this time the skin lesion was only a minute focus of fibroblasts and macrophages.

It may be concluded from these changes that from the standpoint of cellular pathology it appears that antibody formation in this primary phase is quite complicated and that it is in all probability a pluricellular reaction involving several systems of cell types.

It is deduced that granulocytes, lymphoblasts, large lymphocytes,

and possibly basophilic lymphocytes (plasma cells) play an important part in the primary phase of antibody formation.

I wish to acknowledge the many helpful suggestions made by Dr. F. D. Gunn.

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[*Illustrations follow*]

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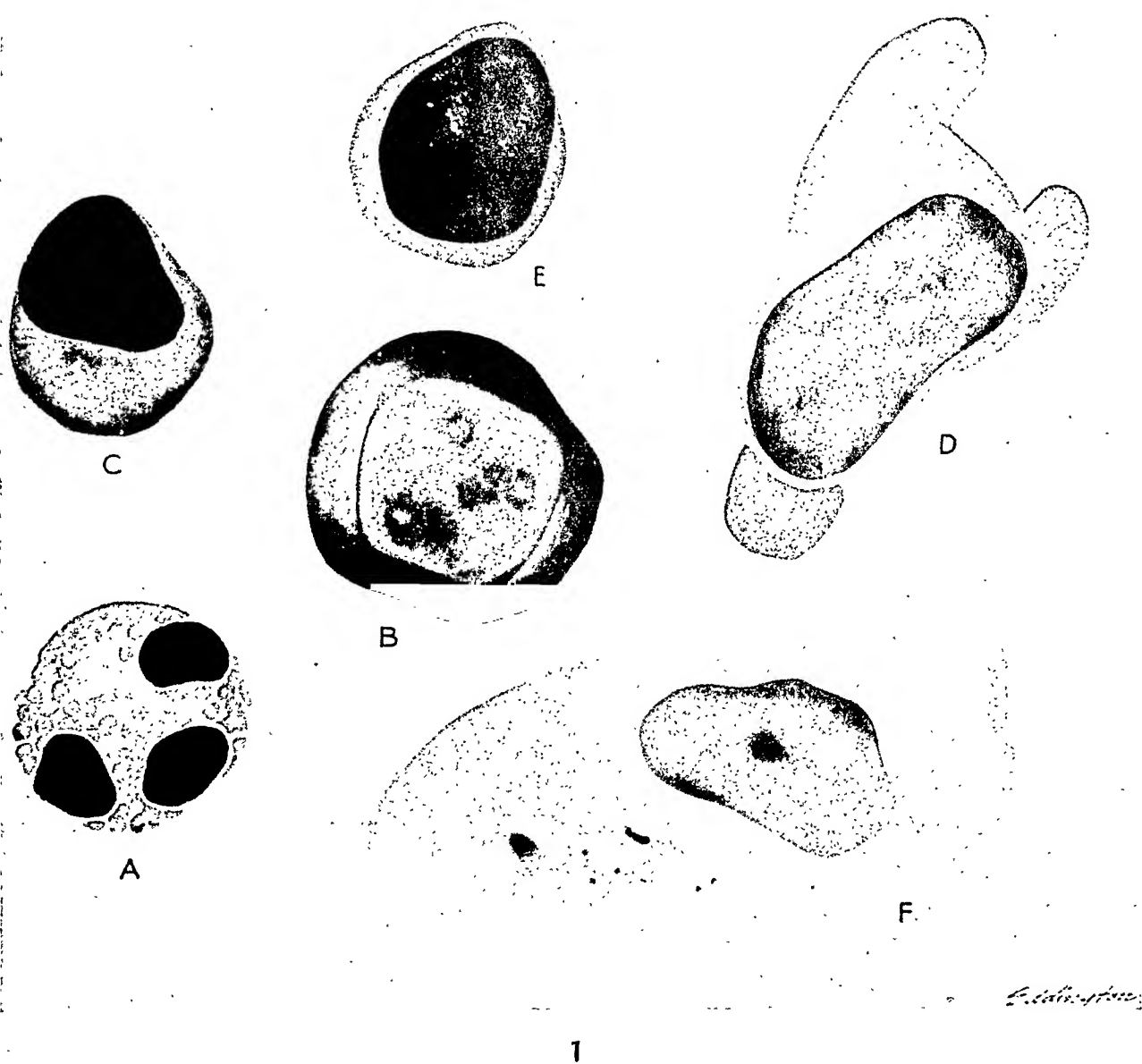
PLATE 209

All plates are drawings of microscopic fields by Keith Eddington.

FIG. 1. Reproduced from colored drawing illustrating the six classified cell types from lymph node smears.

- A. Pseudo-eosinophilic granular leukocyte which is rarely seen in the normal smear.
- B. Lymphoblast with nucleoli.
- C. Basophilic lymphocyte.
- D. Large lymphocyte.
- E. Small lymphocyte.
- F. Reticulum cell.

See text for discussion and differential counts.



McNeil

Cellular Changes During Antibody Formation

PLATE 210

FIG. 2. There is a diffuse granular leukocytic infiltration of the corium. Lymphatics are dilated and cellular elements crowd these spaces. Time interval after antigen injection, 12 hours. Hematoxylin and eosin stain. $\times 100$.

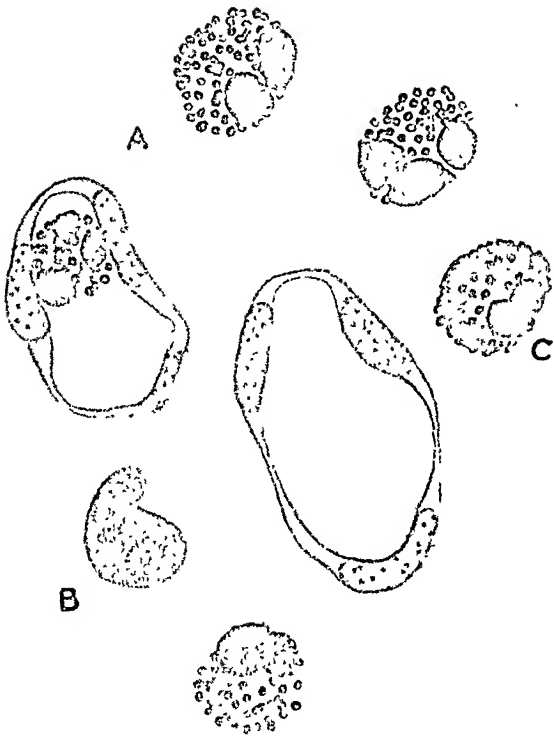
FIG. 3. High-power drawing of cell types seen at site of arrow in Figure 2. Capillaries are dilated and pseudo-eosinophils (A) are numerous. A fair number of true eosinophils (C) and rare neutrophils (B) are present. No bacteria are seen. Hematoxylin and eosin stain. $\times 1000$.

FIG. 4. Low-power view of central zone of right cervical lymph node 24 hours after injection. Many areas of hyperemia (A) are seen and also dilated clear spaces (B) which appear to be lymphatic channels, some of which contain lymphocytes. Hematoxylin and eosin stain. $\times 100$.

FIG. 5. High-power view illustrates cell types seen in right lymph node at 24 hours before lymphoblastic activity commences. Pseudo-eosinophils and eosinophils (A) appear in sinuses and a few large lymphocytes (B) are nearby. Some of the eosinophilic cells are disintegrating (C). Endothelial cells (D) and reticulum cells appear undisturbed. Small lymphocytes are scattered about.



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THROMBOTIC (EMBOLIC) AORTIC OCCLUSION ITS OCCURRENCE AMONG MENTALLY SICK PATIENTS *

GEORGE S. STRASSMANN, M.D.

(From the Laboratory of the Metropolitan State Hospital, Waltham, Mass.)

Occluding emboli and/or thrombi of the aorta are considered to be rare. In general hospitals they are found in about 0.12 per cent of all autopsies.¹⁻⁵ In the autopsy material of mental hospitals small aortic thrombi are a common finding in elderly patients, and obliterating aortic thrombi are not rare. An explanation was sought for the high incidence of aortic thrombi among mentally sick patients.

MATERIAL

During a 4-year period, 382 autopsies were performed on psychotic patients. Aortic thrombi of varying size were found in 57 cases (18 males and 39 females). Psychoses of all types were responsible for the admission of these patients to the institution, but senile and arteriosclerotic psychoses occurred in 50 per cent of the cases. According to the size and the nature of the thrombi, the 57 cases were divided into three groups. The largest group (I) consisted of 37 cases with small adherent thrombi.[†] The smallest group (II) comprised 7 cases of aortic aneurysm, 6 of which were of arteriosclerotic origin and showed thrombi inside and outside the aneurysm. Group III consisted of 13 cases with large aortic thrombi which obliterated the lumen of the vessel.

The fatal disease varied. Bronchopneumonia, pulmonary embolism, myocardial infarction, coronary arteriosclerosis and thrombosis, cerebral thrombosis and hemorrhage, and severe enterocolitis were the main causes of death. Wasting diseases such as cancer or caseous tuberculosis were rare. The nutritional condition and the ages of the patients can be seen in Tables I and II.

Group I

Small adherent thrombi were found in the aortas of the 13 men and 24 women comprising group I. Nutritional condition and age are shown in Tables I and II, respectively. The psychoses for which the patients had been treated were of different types (mental deficiency, schizophrenia, paranoid state), but arteriosclerotic psychoses were predominant. The thrombi were found over or near atheromatous plaques and they were always situated in the abdominal aorta near the bifurca-

* Received for publication, November 26, 1947.

† After conclusion of the study, 10 more cases (5 men and 5 women in the age between 65 and 96) showed small nonobliterating thrombi of the aorta.

tion. In 14 cases thrombi were found also in the descending thoracic aorta, and in 8 cases in the arch and the ascending aorta. None of these thrombi had occluded the lumen. The fatal disease varied; bronchopneumonia was the most common cause of death. In addition to atheromatosis of the aorta, sclerosis of the heart valves, and arteriosclerosis of the coronary, renal, and cerebral arteries were usually present.

Group II

Aortic aneurysm was relatively rare in this material. Only one large syphilitic aneurysm of the ascending aorta was observed. This was found in a 57-year-old man, hospitalized for 30 years with a diagnosis

TABLE I
Distribution of Cases as to Nutritional State

	Total	Poor	Moderate	Obese
Total	57	28	12	17
Group I	37	18	9	10
Group II	7	4	2	1
Group III	13	6	1	6

TABLE II
Distribution of Cases According to Age

	31-40 years	41-50 years	51-60 years	61-70 years	71-80 years	81-90 years	Over 90 years	Total
Total	1		5	8	21	20	2	57
Group I			2	4	15	15	1	37
Group II			1		3	2	1	7
Group III	1		2	4	3	3		13

than 50 years. She was a well nourished mentally deficient woman, 33 years old. The cause of her aortic thrombosis was polycythemia. The same underlying pathologic condition was responsible for the thrombosis found in 2 other women, 72 and 80 years old. In the 72-year-old woman the thrombi were situated in the arch of the aorta and had extended into the right innominate, subclavian, and carotid arteries, causing gangrene of the right arm. In the other 12 cases the thrombi were situated in the abdominal aorta and extended upward from the bifurcation over a varying area and downward into one or both iliac arteries and their branches. In 4 cases the orifice of one renal artery was more or less occluded by the thrombus, resulting in large infarcts and atrophy of the kidney. In one case the orifices of both mesenteric arteries were occluded and terminal intestinal gangrene was found. Total obliteration of one or both iliac arteries had caused gangrene of one or both legs shortly before death in 9 instances. The fresh obliterating thrombi were soft and dark. In 4 cases, long-standing thrombi, silent for an undetermined period, were found firmly attached to the aortic wall, appearing as mushy, grayish yellow clots. Microscopically, these thrombi were homogenously pale-staining with only a few cellular elements in them. A fibrinoid layer separated them from the proliferated intima. There were a number of histiocytes with hemosiderin in the intima and numerous round-celled accumulations in the intima, media, and especially in the adventitia, indicating a reaction to thrombosis. Patients with senile and arteriosclerotic psychoses, mental deficiencies, schizophrenias, and alcoholic psychoses were found in this group.

From the presence of mural thrombi in the distended left auricles of 2 women with chronic mitral valvulitis and mitral stenosis, it was concluded that the obstruction was primarily embolic, followed by secondary thrombosis. One was a 55-year-old schizophrenic, the other a 68-year-old arteriosclerotic patient. An embolic origin appeared possible in 2 cases with coronary occlusion, myocardial infarction, and mural thrombi in the left ventricle and in one case with a distended left auricle containing mural thrombi, and with coronary and generalized arteriosclerosis. However, formation of the clot by thrombosis could not be excluded. In 8 other cases without mural thrombi in the heart, primary thrombosis at the bifurcation was probable. Included are the 3 cases of polycythemia in which an increased tendency to clotting existed during life. In the remaining cases atheromatosis of the aorta and impairment of the blood flow by coronary sclerosis, myocardial infarction, or calcific valvulitis were the main reasons for the formation of the thrombus. Ten of the 13 patients were over 60 years of age and showed arteriosclerotic heart disease and/or generalized arterioscle-

rosis. Atrophy of the organs was common, as is usual in elderly psychotic patients.⁷ Weight of the brain varied from 950 to 1050 gm.; of the spleen, from 50 to 100 gm.; of the liver, from 860 to 1050 gm.; and of one kidney, from 50 to 100 gm. The heart showed usually a moderate hypertrophy with an average weight of about 400 gm. In only a few cases, as in the patients with polycythemia or in very obese patients, was there encountered a brain weighing 1400 gm., a spleen weighing 250 gm., a kidney weighing 210 gm., a liver weighing 1880 gm., or a heart weighing 600 gm.

One of the cases was unusual because the silent thrombosis extended from the isthmus of the aorta to the bifurcation, occluding the left renal artery and extending into both iliac arteries. The patient, a 72-year old, well-nourished woman with an alcoholic psychosis, had been hospitalized for many years and had been treated for diabetes. Signs of the thrombosis developed only 2 days before death when the right iliac artery became wholly obstructed and gangrene of the right foot developed. Thrombosis of the left renal artery had resulted in total necrosis of that kidney with superimposed suppuration, but no evidences of renal damage had been observed during life. The gross and microscopic picture of the necrotic kidney was different from that of necrotizing papillitis observed in diabetic patients.⁸⁻¹⁰

cent of all autopsies (57 of 382 autopsies). Obliterating thrombi were found in 3.4 per cent of the autopsies (13 of 382 autopsies). Neither the type of psychosis, which varied to a large degree, nor the nutritional condition was an important factor. Emaciated and obese patients showed the same tendency to the formation of small mural or obliterating aortic thrombi. It may be that some of the patients were better nourished before the onset of their mental or physical disease, but a number of the emaciated patients had been hospitalized for a long period and no chronic wasting diseases were found to be responsible for the emaciation. Mental disturbances, the difficulty in feeding, the slowing of metabolism, and the generalized arteriosclerosis explained the poor nutritional state of the patients.⁷ The fatal terminal disease was too variable to account for the frequency of aortic thrombi. This frequency is probably due to the fact that 254 of 382 autopsies, or 66 per cent, were performed on patients over 60 years of age and that 160 of 382 autopsies, or 42 per cent, were performed on patients over 70 years of age, in whom more or less advanced atherosclerosis, especially in the abdominal aorta, was present. Elderly patients form a large proportion of the inmates of mental hospitals at the present time, and it is probable that the number of these patients will increase with the ageing of the whole population. Although obliterating thrombi may remain silent for some time, it seems likely that some of the thrombi were found incidentally at necropsy only because the confused and mentally deteriorated patients did not complain before stormy symptoms and gangrene had developed. Many of these patients had been admitted to the hospital because of mental symptoms which were due to impairment of the cerebral circulation and to cerebral arteriosclerosis. It is understandable that these patients often showed other vascular disturbances on the same basis, such as coronary disease, myocardial infarctions, calcific valvulitis, aortic atheromatosis, and aortic thrombi.

SUMMARY

Small aortic thrombi in atheromatosis of the aorta are frequent in mentally sick patients over 60 years of age. Thrombotic aortic occlusions among this special group are not as rare as would be concluded from the autopsy material of general hospitals. In the material utilized for this study, aortic thrombosis was found in 57 of a series of 382 autopsies. In 13 cases the thrombus was obturating. The reason for the greater frequency in the mentally sick group is, apparently, the more advanced average age of this group.

Since submitting this paper for publication, 6 more cases of aortic thrombosis have been observed. The patients were from 71 to 91 years old and had signs of advanced sclerosis of the aorta, coronary arteries, and heart valves. In 2, gan-

grene of the legs, and in one, gangrene of the intestines, had developed shortly before death. In one instance the thrombus was situated at the isthmus. In the other 5 cases it was found at the bifurcation and extended into the iliac arteries.

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